Enhancing the biological control of leafrollers (Lepidoptera: Tortricidae) using floral resource subsidies in an organic vineyard in Marlborough, New Zealand

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By

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Enhancing the biological control of leafrollers (Lepidoptera: Tortricidae) using floral resource subsidies in an organic vineyard in Marlborough, New Zealand

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Abstract

In this thesis, experiments were conducted in both the laboratory and the field to determine whether the provision of floral resources to *Dolichogenidea tasmanica* Cameron (Hymenoptera: Braconidae) could enhance the biological control of leafrollers (Lepidoptera: Tortricidae) in an organic vineyard in Marlborough, New Zealand. Laboratory experiments were conducted to find a selective floral resource that could enhance the 'fitness' of the parasitoid, *D. tasmanica* without enhancing that of its host, the lightbrown apple moth, *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae). From these experiments it was found that the longevity of adult female and male *D. tasmanica* could be enhanced significantly from 2.8 and 3.8 days, respectively, with water. to 18.4 and 12.4 days, respectively, with buckwheat, *Fagopyrum esculentum* Moench, cv. Katowase. Also, adult *E. postvittana* 'fitness' was not enhanced when exposed to buckwheat and first-instar *E. postvittana* larvae 'preferred' grapevine to buckwheat leaves. Therefore, buckwheat was tested for its ability to enhance the biological control of *E. postvittana* in the vineyard. In field experiments in 2003, leafroller parasitoids were more abundant in areas of the vineyard planted with buckwheat and greater parasitism rates of naturally-occurring leafroller larvae were recorded in vineyard plots with buckwheat compared with control areas. In 2004, field experiments showed that rubidium chloride could be used

to mark parasitoids feeding on buckwheat nectar and that *D. tasmanica* dispersed at least 30 m from buckwheat plants within a seven-day sampling period following feeding. Also, parasitism rates of leafroller larvae were greater adjacent to the buckwheat (41 %) than at 10 m from it (19 %). In a large-scale field experiment conducted in 2005, parasitism rates of naturally-occurring leafroller larvae were again found to be greater in areas of the vineyard planted with buckwheat and there were fewer larvae in grape bunches at harvest time in buckwheat compared with control areas. Therefore, the results of this work indicate that buckwheat may be used as a "selective food plant" to enhance the biological control of leafrollers in New Zealand vineyards. Future work could further explore whether buckwheat can reduce leafroller larvae in grape bunches to below economic thresholds, as this result is more likely to encourage grapegrower uptake of this technology.

Keywords

Dolichogenidea tasmanica, leafroller, buckwheat, floral resource subsidies, conservation biological control, habitat manipulation, *Epiphyas postvittana,* grapes.

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Chapter 1 Introduction

1.1 Conservation biological control

Since the 1940s, the most common method of controlling insect pests has been through the use of pesticides (Hajek, 2004). Pesticides are usually extremely effective at killing insect pests; however, there are many negatives associated with their use including; resistance, target pest resurgence, secondary pest outbreaks, environmental pollution, hazards to human health and negative effects on natural enemies (Hajek, 2004). It was not until the 1960s that the integrated pest management (IPM) concept was developed in response to concerns about the impacts of pesticides on the environment. Initially, this concept embraced the combined use of natural enemies and pesticides to manage pests (Stern et al., 1959). However, over recent decades, IPM has evolved to include the combined use of multiple pest-control methods, such as cultural control, host plant resistance, biological control and the use of chemical control (Gurr et al., 2004). Of these methods, biological control, which may be defined as "the action of parasites, predators or pathogens in maintaining another organism's population density at a lower average than would occur in their absence" (DeBach, 1964) has become a popular method of managing arthropod pests. This can be accomplished by either: (1) classical biological control, which is the importation of exotic enemies against either exotic or native pests (Ehler, 1998); (2) augmentative biological control, which involves the direct manipulation of established species' populations by insectary mass production and periodic colonisation (Debach & Rosen, 1991); (3) conservation biological control, which involves the use of habitat manipulation techniques to modify the environment to protect and enhance natural enemy populations so that they are more effective at managing the target pest

organism (Debach & Rosen, 1991) and so as to minimise pesticide-induced mortality (Gurr & Wratten, 2000). Conservation biological control differs from classical and augmentative approaches as natural enemies are not released. Instead, natural enemy populations that already exist in or near the area are conserved or enhanced.

As previously mentioned, CBC involves the use of habitat manipulation techniques; however, the two approaches are derived from different hypotheses (Fig. 1.1). Habitat manipulation employs techniques of cultural control such as crop diversification, which is common to CBC, the difference being that the plant protection that results from habitat manipulation is a more 'bottom-up' (first trophic level) mediated approach. This stems from the 'resource concentration' hypothesis (Root, 1973), whereby pest suppression occurs as a result of non natural-enemy effects, for example, by the crop being 'diluted' by cues from other plants. However, CBC also encompasses habitat manipulation by providing natural enemies with resources such food (in the form of nectar (Baggen & Gurr, 1998), pollen (Hickman & Wratten, 1996) or homopteran honeydew (Wäckers, 2000)), shelter (Halaji et al., 2000) or alternative prey or hosts (Viggiana, 2003). When herbivores are suppressed by natural enemies in this manner, control is said to be occurring via 'top-down' (third trophic level) approaches and supports the 'natural enemies' hypothesis (Root, 1973).

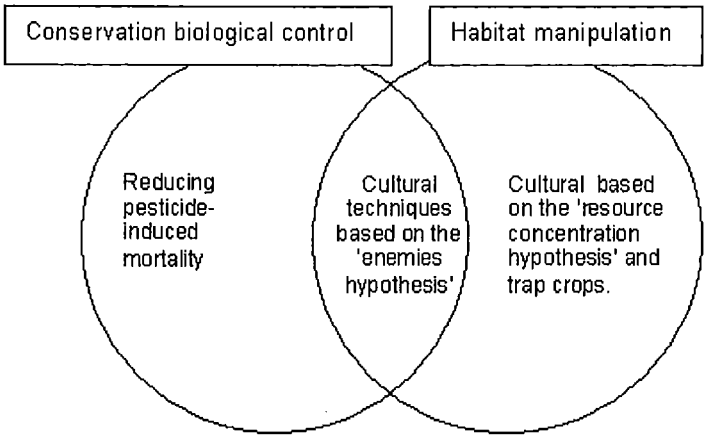

Fig. 1.1 Comparing and contrasting habitat manipulation and conservation biological control approaches to pest management.

From Gurr, G.M., Scarratt, S.L., Wratten, S.D., Berndt, L., & Irvin, N. 2004. Ecological engineering, habitat manipulation and pest management. In: Ecological engineering for pest management: advances in habitat manipulation for arthropods (G.M. Gurr S.D. Wratten & M.A. Altieri, eds), p. 3, figure 1.3.

Providing shelter and alternative prey or hosts

In modern agricultural landscapes, crops may often consist of a single species (monoculture) and resources such as food, shelter and alternative hosts or prey are rarely available. Therefore, it may be necessary to provide these resources at the correct temporal and spatial scales to enhance natural enemy populations. Crops can be modified in a number of ways to preserve and enhance natural enemies. One of the most successful applications of CBC is the establishment of 'beetle banks' or 'predator conservation strips' (Thomas & Wratten, 1988; Thomas et al., 1991; MacLeod et al., 2004) to provide long-term shelter for natural enemies. Beetle banks are areas of raised earth beds sown with several different grasses to create island habitats for predators within and adjacent to the cropping system (Thomas et al., 1991). In the winter, these areas provide a refuge and higher densities of predatory arthropods are found on them. In spring, beetles and other natural enemies emigrate

from the beetle banks and colonise the crop, potentially preventing aphid outbreaks (Thomas et al., 1991).

Habitat manipulation techniques may also be used to provide alternative prey or hosts to natural enemies, as some natural enemies may require alternative host species in order to overwinter (Doutt & Nakata, 1973; Wilson et al., 1989) or for periods when the target arthropod populations are low. Alternative prey or hosts are often provided through habitat diversification. One example of such diversification is the use of weed strips as refuges for non-crop aphids to provide a host reservoir for aphid natural enemies (Nentwig et al., 1998). This provision of alternative prey or hosts is important when crop aphid populations are low and is a good example of how habitat manipulation techniques may be used to enhance the control of aphids. However, habitat diversification may not always lead to the provision of a suitable alternative prey or host. Powell & Zhang (1983) showed that although the polyphagous aphid parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) parasitises the cereal aphid (*Metopolophium dirhodum*, Wlk.), the pea aphid (*Acyrthosiphon pisum*, Harris) and the nettle aphid (*Microlophium carnosum*, Buckt.) in the field, it did not respond to the nettle aphid or nettle leaves in laboratory experiments, suggesting that there may be more than one race or 'biotype' of the parasitoid and that using nettles as habitat for an alternative host for *A. ervi* in integrated control programmes may not lead to enhanced biological control of cereal aphid populations.

Food plants

A common technique used in CBC is the incorporation of flowering plants into agro-ecosystems to enhance the populations and 'fitness' (longevity and fecundity) of

natural enemies by providing them with resources which may have been previously absent or scarce in that system (Baggen & Gurr, 1998; Tscharntke, 2000). Flowers may provide important resources such as food (nectar, pollen and/or homopteran honeydew) to natural enemies but they may also act as a shelter for natural enemies or provide habitat for alternative prey or hosts (Landis et al., 2000).

Since the early 1900s it has been recognised that adult parasitoids feed on plant-based food sources in the field (Johnston, 1913), such as floral and extra-floral nectar and hompoteran honeydew (Jervis et al., 1992; Wäckers, 1994; Jervis et al., 1996; Stapel et al., 1997; Irvin, 1999; Rivero & Casas, 1999; Olson et al., 2000; Berndt, 2002; Lee et al., 2004). These plant-based foods provide carbohydrates in the form of sugars to adult parasitoids (Wäckers, 2001), in turn providing a much needed source of energy for these insects (Jervis et al., 1993).

As well as providing an energy source, the provision of sugar can markedly improve adult parasitoid fitness, by increasing longevity (Foster & Ruesink, 1984; Jervis et al., 1992; Wäckers & Swaans, 1993; Heimpel et al., 1997; Baggen & Gurr, 1998; Olson et al., 2000; Berndt, 2002; Costamagna & Landis, 2004; Berndt & Wratten, 2005; Irvin et al., in press) and fecundity (Baggen & Gurr, 1998; Berndt, 2002; Irvin et al., in press). Therefore, introducing flowering plants into the cropping system could increase parasitism rates by increasing parasitoid longevity and fecundity.

In field experiments, parasitism rates increased significantly when flowering plants were present (Leius, 1967; Foster & Ruesink, 1984; Stapel et al., 1997; Baggen & Gurr, 1998). For example, parasitism rates of the potato tuber moth, *Phthorimaea*

operculella Zeller (Lepidoptera: Gelechiidae), were significantly increased by the parasitoid *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtida) in a potato crop when dill and borage were added to the system (Baggen & Gurr, 1998). Also, rates of parasitism of the tent caterpillar, *Malacosoma americanum* (F.) (Lepidoptera: Lasiocampidae) and codling moth, *Carpocapsa pomonella* L. (Lepidoptera: Tortricidae) were higher in apple orchards where wild flowers were present as undergrowth compared with orchards with poor floral undergrowth (Leius, 1967).

Although there are a number of examples which demonstrate the successful implementation of CBC, many of the published studies do not demonstrate reductions in pest damage (Gurr et al., 2000; Heimpel & Jervis, 2005). Fewer of these studies conclusively show that it is the nectar feeding by parasitoids which has led to increased levels of parasitism. Therefore, there is a need for more directed studies which show that the provision of food sources in agricultural systems and the direct feeding on these resources by natural enemies leads to increased biological control in those systems.

Gurr et al. (2003) listed five steps in a hierarchy of successful implementation of CBC. These steps may be used to determine whether successful conservation biological control has been achieved. They are:

1. Aggregation of natural enemies at or near the flowers
2. An enhancement of the natural enemies' 'fitness' (longevity, fecundity and searching efficiency)
3. An increase in parasitism or predation rate in the pest population
4. A decrease in pest population density

5. The pest populations are brought below the relevant economic threshold (so avoiding the need to apply curative insecticides).

However, these steps do not take into consideration which mechanism is attracting the natural enemies to the floral resources (ie. food, shelter and / or alternative prey or hosts) (Landis et al., 2000). In a recent review, Heimpel and Jervis (2005) used the term "parasitoid nectar provision hypothesis" to suggest that biological control of pests will be improved through the presence of nectar-producing plants that supply parasitoids with sugar. They suggest that similar outcomes as the hierarchy of success (Gurr et al., 2003) will occur if the incorporation of flowering plants successfully enhances biological control. However, they suggest that it is not parasitoid aggregation which is important but that it is more important to validate that the mechanisms leading to nectar-mediated improvement of biological control actually occur in the field and that the magnitude of the effect is enough to drive pests below economic thresholds. Heimpel and Jervis' (2005) requirements for validating the parasitoid nectar provisioning hypothesis are:

1. Sugar limitation in parasitoids
2. Feeding on floral nectar in the field
3. Enhanced fecundity by female parasitoids that use nectar
4. Increased parasitism rates and decreased pest densities in the presence of nectar.

Therefore, in this thesis, the hierarchy of success (Gurr et al., 2003) and the requirements of the parasitoid nectar provisioning hypothesis (Heimpel & Jervis, 2005) are examined experimentally in the laboratory and field through the provision

of selective floral resources to enhance the biological control of leafrollers (specifically *E. postvittana*), where selective floral resources enhance the fitness of the natural enemy, without enhancing the fitness of the pest (Baggen & Gurr, 1998).

1.2 *Vitis vinifera* and the New Zealand wine industry

The cultivation of the wine grape, *Vitis vinifera* and the production of wine is believed to date back to Pharaohs of Egypt at least five or six thousand years ago (Jackson & Schuster, 1994). Since then, grapegrowing and winemaking spread throughout the Mediterranean into North Africa, and more recently, European exploration and colonisation has spread viticulture into all of today's wine-producing countries. It was not until the early nineteenth century that vines were established in these countries (Jackson & Schuster, 1994) and although the establishment of vines in Australia and New Zealand came much later than in Europe, they now supply approximately 10% of the world's wine production (Jackson, 2000).

New Zealand's vineyard area has almost tripled in the last decade, with over 18 000 hectares of wine grapes now planted (www.nzwine.com, Gurnsey et al., 2004). Consequently, New Zealand wines are more widely available to export markets (Anderson, 2004) and as a result of increased competition with overseas markets, new and innovative methods of marketing these wines have been necessary. This has led to New Zealand wines being internationally promoted as "the riches of a clean, green land". This "green" image is not only important for exports but also as local consumers are becoming increasingly aware of the processes of wine production. To maintain this image, the New Zealand Integrated Winegrape Production (NZIWP) scheme was established in 1995 and rebranded in 2002 as Sustainable Winegrowing

New Zealand® (SWNZ). It provides a framework for environmentally and economically sustainable viticultural practices. It promotes the philosophy that vineyard practices should have an increased awareness of environmental sustainability and should maintain such sustainability by regularly monitoring vines, by the selective use insecticides and by increasing plant diversity within and around the vineyard to promote the establishment of beneficial insects into the system (Crosse, 1998). The SWNZ programme has been adopted on over 60% of the producing area, with a national membership of 403 vineyards (Gurnsey et al., 2004).

1.3 Insect pests and associated natural enemies in New Zealand vineyards

Compared with other parts of the world, there are relatively few serious insect pests in New Zealand vineyards. For example, New Zealand is currently free of the glassy-winged sharpshooter (*Homalodisca coagulata* (Hemiptera: Cicadellidae)) and other leafhopper species, which vector the bacterium *Xylella fastidiosa*, the causal agent of Pierce's disease which now threatens California's wine grape production (Redak et al., 2004). Most insect pests in New Zealand vineyards are generalists, which feed on a number of horticultural crops and include leafrollers, mites, mealybugs and thrips (Charles, 2002; Wratten & Tylianakis, 2002). Mealybugs are considered to be a serious pest to the New Zealand wine industry as they transmit grapevine leafroll-associated viruses, which can reduce vine productiveness (Jordan, 1993). Leafrollers are considered to be significant grape pests in New Zealand as they can cause significant damage (see Section 1.3.2).

1.3.1 Leafrollers

There are six species of leafrollers (Lepidoptera: Tortricidae) which are considered to be significant pests of grapes and other berry fruit crops in New Zealand (Harris, 1994). They are the black lyre leafroller, *Cnephasia jactatana* (Walker), two species of greenheaded leafroller, *Planotortrix octo* (Dugdale) and *P. excessana* (Walker), two species of brownheaded leafroller, *Ctenopseustis obliquana* (Walker) and *C. herana* (Felder and Rogenhofer) and the lightbrown apple moth, *Epiphyas postvittana* (Walker). With the exception of *E. postvittana*, which originates from Australia (Danthanarayana, 1975), all of these species are endemic.

Species abundance and pest status vary geographically and depending on the crop, such that in the North Island of New Zealand *C. obliquana* is the most abundant leafroller species, whereas in the South Island *E. postvittana* is considered to be the most important pest of pome fruit, grapes and other horticultural crops (Scott, 1984).

1.3.1.1 *E. postvittana* biology

Adult moths are pale brown and males have dark brown markings on the hind portion of their forewings. The moths have a wingspan of approximately 10 mm, with males being significantly smaller than females. After mating, the female lays egg masses (ranging from 4-77 eggs) on the upper surfaces of vine leaves throughout the moth's lifespan of approximately 10-14 days (Danthanarayana, 1975). The egg masses are inconspicuous, as the eggs are small and yellow to light green in colour. As the eggs age they become darker, turning a dark brown to black prior to hatching (Morris, 1966).

After hatching, the young larvae disperse, either by crawling or by spinning down on a silken thread until they find a suitable feeding site (Geier & Briese, 1980). They prefer a growing point of lateral growth or a site adjacent to a leaf vein on the underside of the leaf. The neonate larvae spin protective silken tents to cover themselves whilst feeding, but later instars roll or web leaves together, or make nests amongst clusters of fruit (Bucchanan & Amos, 1998). This feeding behaviour, characteristic of leafrollers, makes conventional control extremely difficult, as chemical sprays targeting the leafroller often do not contact the target organism. Larvae remain feeding in these enclosed areas on the vine and pass through six instars until they pupate (Danthanarayana, 1975).

The pupae are green and soft when newly formed, turning brown and hard as they age. The average size is 2.6 mm by 7.6 mm for males and 2.9 mm by 9.8 mm for females (Danthanarayana, 1975). The pupal stage lasts for about two weeks and after the adult has emerged, pupal cases are often found where larval feeding occurred.

In New Zealand *E. postvittana* has four generations per year in northern regions and three generations in the southern North Island and northern South Island (Lo & Murrell, 2000).

1.3.2 Damage

E. postvittana is highly polyphagous and has been recorded as having over 250 host plant species in New Zealand (Suckling et al., 1998). *E. postvittana* infestations can cause significant direct and indirect damage to grape production. The overwintering generation that colonise vines at budburst cause direct damage by feeding on new

shoots or flowers, berries and stalks (Lo & Murrell, 2000). However, unless *E. postvittana* abundance is high at this stage, damage does not usually warrant control. Damage may also occur from late spring to summer, when larvae feed on berry bunches, causing direct losses in grape yield (Bailey, 1997). However, the most significant damage caused by *E. postvittana* is the indirect damage caused by the transmission and spread of the fungus, *Botrytis cinerea* Pers. by the larvae amongst grape bunches (Nair et al., 1988; Bailey, 1997) or by providing infection sites for botrytis fungus by larval feeding on the bunches (Nicholas et al., 1994). Such damage may cause significant losses in grape production; for example in New Zealand, mid-season losses, as a result of botrytis infections, may exceed 20% under favourable conditions, and complete losses of crops can occur before harvest in very wet seasons (Nicholas et al., 1994).

In an Australian study, Buchanan (1977) demonstrated that 19% of grapes at harvest were damaged by *E. postvittana* infestations. Even though this percentage is high, it was considered to be an underestimate, as berries damaged earlier in the season were excised and were excluded from harvest counts. In New Zealand, Lo and Murrell (2000) introduced *E. postvittana* larvae into grape bunches at monthly intervals to determine the percentage weight loss caused by certain infestation levels. They then calculated that infestations of 5% and 30% of bunches were equivalent to weight losses of 0.6% and 3.6% respectively.

1.3.3 Methods of control

Commonly, *E. postvittana* and other leafroller species are controlled using synthetic pesticides (Scott, 1984; Lo et al., 2000). Although pesticides are an effective way of reducing pest populations, there are a number of negative factors associated with their use. These include the indirect removal of non-target organisms such as natural enemies from the system, pollution of the environment (Samways, 1994) and evidence that pesticides can lead to resistance in the pest species. *E. postvittana* exhibited resistance to insecticides as long ago as 1961/62 (Collyer & Geldermalsen, 1975). Also, in organic vineyards, such as the one in which this project was based, artificial chemicals are excluded from the system, so establishing alternative methods of pest control are even more critical to maintain low pest populations. Alternative methods for controlling leafrollers include the use of pheromone disruption, *Bacillus thuringiensis* (*Bt*) sprays as well as using arthropod biological control agents.

Managing leafrollers through the use of biological control agents involves the use of insect natural enemies (predators and parasitoids) and / or pathogens to reduce leafroller densities to a level lower than would occur in their absence (Bugg & Pickett, 1998).

1.3.4 Natural enemies of *E. postvittana*

E. postvittana is attacked by a wide range of predators and parasitoids in New Zealand during most of its developmental stages. Typically, insect predators which attack leafrollers include earwigs, ladybird beetle adults and larvae, spiders, lacewing larvae and predatory wasps (Baker et al., 1994; Wearing & Harris, 1999; Miliczky & Calkins, 2002; Lucas et al., 2004). Although there has been little work conducted on

predators of leafrollers in New Zealand, a recent study in the United States showed that predation of another tortricid species, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) was greater in areas of an apple orchard where habitat manipulation techniques had been employed, compared with areas of herbicide treated habitat (Matthews et al., 2004). Recent work in New Zealand using living leafroller larvae pinned to vine or soil substrates, showed that predation of leafroller larvae was 50-60% per night and that the most effective predators were earwigs (Frank, unpublished data).

In New Zealand, there has been a comprehensive body of work which has examined leafroller parasitoids and the effects of habitat manipulation on them. Thomas (1989) provided a comprehensive list of parasitic Hymenoptera which attack *E. postvittana*, of which those in the families Trichogrammatidae and Braconidae are the most common. The biology of some of the most common parasitoids of *E. postvittana* is provided below. Thomas (1989) stated that the other parasitoid families generally occur less frequently but may be of sporadic or local importance.

Species within the Trichogrammatoidea, including *Trichogramma (Trichogrammanza) funiculatum* (Carver) and *Trichogramma bactrae fumata* (Nagaraja), are solitary endoparasitic wasps which parasitise the eggs of *E. postvittana* and other tortricid species. Trichogrammatid wasps are the only natural enemies known to attack the eggs of leafrollers in New Zealand (Thomas, 1989). Thomas (1989) recorded parasitism rates of leafroller egg batches by trichogrammatid species in a citrus orchard (Nelson, New Zealand) and found that parasitism rates rose to 90% in the Autumn of 1969-1970, but were less than 10% in spring. This high

variability of parasitism rates suggests that although these species may exhibit high parasitism rates during certain periods of the year, they may not have a high impact factor on leafrollers during other times of the year when control is important. However, these wasps are used extensively in Australia for innundative releases for leafroller control (Glenn & Hoffmann, 1997).

Dolichogenidea tasmanica Cameron (Hymenoptera: Braconidae) has been recorded as the most common parasitoid to attack its primary host, *E. postvittana* (Charles et al., 1996; Suckling et al., 1998; Berndt, 2002). However, although it is an important species in the biological control of leafrollers in New Zealand, there is not a lot of published information about the biology and ecology of this species. It is known that it is a solitary endoparasitoid of leafroller larvae. The female wasp lays a single egg within the 1st or 2nd instar of the leafroller larvae and it continues to develop inside the larva until the larva reaches its 4th instar. The parasitoid larva then emerges from the host to produce a white cocoon. This species is originally from Australia, however, its mode of introduction into New Zealand is unknown.

Glyptapanteles demeter (Wilkinson) (Hymenoptera: Braconidae) is native to New Zealand and is a gregarious endoparasitoid of leafrollers. It lays its eggs in the 1st and 2nd instars of leafroller larvae. Development is completed by the time the leafroller reaches its 5th or 6th instar. Between two and 29 wasps are produced from each leafroller larva and when development is completed, groups of white cocoons are found associated with the remains of the leafroller (www.hortnet.co.nz).

Goniozus sp. (Farrugia) (Hymenoptera: Bethylidae) is a gregarious ectoparasite which parasitises the 3rd or 4th instar leafroller larval stages. Its larvae feed externally on the host and are often seen in groups attached to the first abdominal segment of leafroller larvae (www.hortnet.co.nz).

Glabridorsum stokesii (Cameron) (Hymenoptera: Ichneumonidae) was introduced to New Zealand from Australia as part of the 1960s and 1970s biological control programme for the lightbrown apple moth. It lays an egg on the surface of leafroller pupae and the wasp larva feeds externally before becoming an internal parasite. As well as parasitising leafrollers, this wasp is an important natural enemy of Oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) and occasionally attacks codling moth pupae (www.hortnet.co.nz).

1.3.5 Economic thresholds for *E. postvittana*

The presence of insect pests in agro-ecosystems does not always mean that that insect needs to be controlled. This is particularly true of insect pests that occur at low densities without causing a problem. Economic injury levels were developed to determine whether an organism needs to be controlled and are defined as the lowest density of pests that will cause economic damage (Hajek, 2004). An economic threshold is usually set below the economic injury level and once densities of pests reach this threshold, control practices should begin to reduce the possibility of any economic loss.

In Australia, thresholds for the control of lightbrown apple moth in grape vines have been established (Baker, 1999). The levels are more than 10 larvae per 50 shoots or

more than five larvae per 50 bunches (10%). In New Zealand, however, economic thresholds for leafrollers or lightbrown apple moth have not been established. It is, however, recommended in the Sustainable Winegrowing New Zealand (SWNZ) manual that if 1% of bunches are infested or show signs of leafroller damage, at the time of flowering, then control should be implemented. Subsequently, if 2-4% of bunches are infested or show signs of infestation, at bunch closure, control should be implemented, and if 5% of bunches are infested at harvest, insecticides should be sprayed the following spring (Charles, 2002). The thresholds listed in the SWNZ manual are surprisingly low compared to Australian thresholds, since leafrollers do not vector viruses and damage caused by leafrollers is not of any real 'cosmetic' concern, as damaged bunches are heavily processed for wine production. Certainly, leafrollers can cause economic loss to winegrape production, however, these thresholds may be contributing to overuse of chemical pesticides in the "clean, green" New Zealand wine industry and need to be reassessed.

1.4 Levels of success reached using CBC to manage leafrollers

To date, there has been a substantial body of work which has examined the effects of understorey management on the biological control of leafrollers in New Zealand and Australia (Stephens et al., 1998; Berndt et al., 2002; Begum et al., in press; Berndt et al., in press; Irvin et al., in press). In New Zealand, Stephens et al. (1998) and Irvin et al. (in press) examined the effects of understorey management on leafrollers in orchards and Berndt et al. (2002) worked on conservation biological control and habitat management techniques to enhance natural enemy populations of *E. postvittana* in vineyards.

All three of these studies demonstrated that the first level of the hierarchy of success (Section 1.1) could be met when significantly more *D. tasmanica* were collected on yellow sticky traps in buckwheat plots compared with control plots where no flowering plants were present (Stephens et al., 1998; Berndt et al., 2002; Irvin et al., in press). Irvin et al. (in press) examined the effects of buckwheat plants on *D. tasmanica* fitness in laboratory experiments. The results showed that longevity of female *D. tasmanica* was increased from 12 days (water only) to 35 days when they were exposed to buckwheat, and that buckwheat enhanced potential fecundity by 62 % (Irvin et al., in press); thus the second level of the hierarchy was reached. The third level was achieved when parasitism rates of leafroller larvae were increased by more than 50 % in one of three vineyards where buckwheat flowers were present (Berndt et al., in press). At the other two vineyards in that study, buckwheat had no effect on parasitism rates, but at these locations leafroller populations were low because insecticides had been used in that growing season (Berndt et al., in press). This illustrates one of the difficulties inherent in field testing CBC methods within production systems where pesticides are used.

Another potential problem associated with field testing of CBC is inadvertently exacerbating pest problems by adding to the production system a plant that benefits a pest (Baggen & Gurr, 1998). Therefore it is important not only to investigate which food plants can selectively enhance the parasitoid but also which are not exploited by the pest. Of all three studies outlined above, none has tested whether adult *E. postvittana* can feed on and / or benefit from the buckwheat flowers. In an Australian study, Begum et al. (in press) found the longevity of adult female *E. postvittana* to be

as long when provided access to buckwheat flowers as when provided an artificial honey based diet, suggesting that there may be some benefit of the flowers to the pest.

Although the research described here has shown that the first three levels in the hierarchy of research outcomes can be achieved in the *D. tasmanica*-leafroller system, the fourth and fifth levels have not yet been demonstrated.

Also, there is a lack of information on the movement or dispersal of the parasitoid, *D. tasmanica* from floral resources. An understanding of how far parasitoids disperse from floral resources can enable biological control workers to make recommendations on the deployment of such resources in agricultural systems. Therefore, there is a need to measure how far *D. tasmanica* disperses from these floral resources and to measure the spatial scale over which these resources act, as this information may facilitate a greater understanding of whether habitat manipulation techniques lead to increased localised concentrations of natural enemies and whether they contribute to greater suppression of pest populations over a larger spatial scale.

1.5 Measuring spatial dynamics of insects in CBC research

In CBC research, it is important to study the dispersal of natural enemies from the area of habitat manipulation in the cropping system, as that area may occupy only a small proportion of the total crop and the movement of the natural enemy will determine the spatial extent of enhancement. To measure insect movement, markers are often employed. A wide variety of markers and marking techniques has been used to track insect movement (Hagler & Jackson, 2001; Lavandero et al., 2004). Some materials and techniques which have been used to mark insects in the past include:

tagging, mutilation, paint, dust, dye, pollen, genetic, radio-active isotopes and protein marking (Hagler & Jackson, 2001; Lavandero et al., 2004). Several of these techniques have proven useful in marking and tracking parasitic Hymenoptera; however they may also be limited by application and other technical difficulties and consequent changes in insect behaviour.

The use of trace elements such as rubidium is one method which may be used for internally marking and tracking insect dispersal. This method, first proposed by Berry et al. (1972), has since been used in a number of studies to track insect movement (Payne & Wood, 1984; Hopper & Woolson, 1991; Corbett et al., 1996; Fernandes et al., 1997; Prasifka et al., 2001). However, it was not until the work of Freeman-Long et al. (1998) that rubidium was used as a marker to associate the feeding of natural enemies on floral resources in the field and to monitor their movement into adjacent crops.

Freeman-Long et al. (1998) injected or sprayed rubidium chloride (RbCl) in or onto flowering plants and subsequently insects were labelled with this element by coming into contact with the plant, presumably through feeding on the nectar or pollen. Insects were then collected on traps and analysed for their rubidium content. The overall result of this experiment was that beneficial insects fed on nectar or pollen provided by insectary plants and then moved distances of up to 250 feet from the flowering plants, suggesting that flowers may be providing food for insects which travel relatively long distances from the source. Therefore, information on natural-enemy movement, spatial distribution patterns and density are crucial for enhancing parasitoid efficacy at the field level (Jervis et al., 2004).

1.6 Aims

Although there are clear benefits in providing buckwheat as floral resources for *D. tasmanica,* further work is needed to rank buckwheat against other flowering plant species to determine whether there are any other species which may also enhance the biological control of *E. postvittana.* Further research is also necessary to determine the optimal densities for planting buckwheat and other flowering plants in vineyards and to determine the spatial scale over which nectar sources affect parasitoid dynamics. Finally, the effectiveness of buckwheat to enhance the biological control of *E. postvittana* needs to be analysed further (based on the measures of success of Gurr et al. (2003)) and the requirements for validating the parasitoid nectar provisioning hypothesis of Heimpel and Jervis (2005) that may result in increased biological control of the target pest.

Specific aims are:

1. To test a wider range of flowering plant species than previously tested, to determine which species can enhance the fitness of *D. tasmanica* without enhancing that of *E. postvittana* under laboratory conditions.
2. To enhance population density of natural enemies of *E. postvittana* using selective floral resources in the vineyard.
3. To examine parasitism rates of *E. postvittana* and other leafroller species in the presence of selective floral resources in the vineyard.

4. To study *D. tasmanica* dispersal from floral resources in the vineyard, to determine how far this species is dispersing from these resources so that informed decisions may be made on the appropriate spatial scale for these resources to be planted in vineyards to enhance the biological control of *E. postvittana.*
5. To measure parasitism rates and pest abundance in a large-scale field experiment, comparing areas where floral resources are planted with areas where they are absent.

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Chapter 2 Identifying a selective floral resource for conservation biological control of *Epiphyas postvittana*

2.1 Abstract

To find a selective floral resource that could enhance the fitness of the parasitoid, *D. tasmanica* without enhancing that of its host, the lightbrown apple moth, *E. postvittana*, four flowering plant species were tested in laboratory experiments against parasitoid and moth fitness and larval feeding preferences. Buckwheat, mustard, *Brassica rapa* L., bishop's flower, *Ammi majus* L., and dill, *Anethum graveolens* L., were the flowering plant species chosen for use in these experiments. When these species were tested against the fitness of adult *E. postvittana* and *D. tasmanica,* the longevity of adult female *E. postvittana* was not affected by the treatments; however, males had decreased longevity in the no food and no water treatment. The fecundity of adult *E. postvittana* did not differ between treatments. In contrast, the longevity of adult female and male *D. tasmanica* was significantly increased from 2.8 and 3.8 days respectively with water to 18.4 and 12.4 days respectively when provided with buckwheat flowers. Mustard, bishop's flower and dill flowers did not enhance the longevity of either female or male *D. tasmanica.* The potential fecundity of adult *D. tasmanica* was increased when exposed to buckwheat flowers compared with when provided with water only. When first-instar *E. postvittana* larvae were presented with leaves of the above plant species and grapevine (cv. Sauvignon Blanc) in a multiple choice test, grapevine leaves were the most preferred. These results suggest that buckwheat may be used as a 'selective food plant' in the conservation biological control of *E. postvittana* in grapevines.

2.2 Introduction

Conservation biological control involves the use of habitat manipulation techniques to modify the environment to enhance natural enemies (predators or parasitoids) (Debach & Rosen, 1991) and to reduce pesticide use which may negatively impact on natural enemy populations (Gurr & Wratten, 2000). A method commonly used in CBC is the addition of floral resources to a cropping system to provide resources to natural enemies which may have previously been scarce or absent in that system (Landis et al., 2000). Floral resources provide food (in the form of nectar or pollen) to natural enemies and many studies have demonstrated that providing food to natural enemies can enhance their longevity and fecundity (Foster & Ruesink, 1984; Jervis et al., 1992; Wäckers & Swaans, 1993; Heimpel et al., 1997; Baggen & Gurr, 1998; Olson et al., 2000; Berndt & Wratten, 2005). However, care needs to be taken when choosing which type of floral resource to provide, as some may also enhance pest 'fitness' and populations (Zhao et al., 1992; Baggen & Gurr, 1998). Baggen & Gurr (1998) found that the target pest, the potato moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) fed on coriander (*Coriandrum sativum* L.) and faba beans (*Vicia faba* L.) that had been planted in a potato field to enhance the parasitoid *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae). This finding led Baggen & Gurr (1998) to conduct laboratory tests to screen for a 'selective food plant' that would benefit the parasitoid without benefiting the pest. Finally, borage (*Borago officinalis* L.) was found to benefit the parasitoid only, although the mechanisms leading to this selectivity were not understood.

This prompted Baggen et al. (1999) to evaluate the mechanisms which were operating this selectivity. They suggested that it was a combination of the corolla depth,

interference by the stamen appendages and hairs on the style of phacelia (*Phacelia tanacetifolia* Benth.) flowers that prevented *P. operculella* feeding on the nectar whilst allowing *C. koehleri* access. Several other studies have also suggested that floral architecture and parasitoid morphology may determine nectar availability and that measuring the flower aperture and natural enemy morphology may help to screen 'selective food plants' in the laboratory (Patt et al., 1997; Lavandero et al., in press). However, there are several other possibilities that may facilitate such selectivity, such as nectar quality (Galetto & Bernardello, 2004; Steppuhn & Wäckers, 2004) and flower colour (Begum et al., 2004).

In New Zealand the lightbrown apple moth, *E. postvittana,* is one of six species of leafroller that are serious pests of horticultural crops, including apples and grapevines (Scott, 1984). Leafroller larvae damage grapevines by feeding on new shoots, flowers, berries, stalks and leaves (Lo & Murrell, 2000). Indirect damage is caused by the leafroller larvae through the movement of *Botrytis cinerea* Pers. amongst grape bunches (Nair et al., 1988), or by providing infection sites for the botrytis fungus by feeding on the berries and splitting them (Nicholas et al., 1994). Such indirect damage can cause significant losses in grape production. For example, in New Zealand mid-season losses from botrytis infections may exceed 20% under favourable conditions, and complete losses of crops can occur close to harvest in very wet seasons (Nicholas et al., 1994). Leafrollers can be managed with pesticides, but as the New Zealand wine industry is promoting its wines as the 'riches of a clean, green land', alternative methods of pest management are being sought. In New Zealand, leafrollers are attacked by a wide range of predators and parasitoids during most of their life stages (Thomas, 1989). Of these biological control agents, *D. tasmanica* effects the highest

parasitism rates of leafroller larvae in New Zealand (Thomas, 1989; Berndt et al., 2002; Irvin et al., in press) and is an important natural enemy of these pests. *D. tasmanica* benefited from feeding on floral resources, such as alyssum (*Lobularia maritima* L.) and buckwheat (Irvin et al., in press). However, as alyssum was found to increase adult *E. postvittana* longevity and fecundity (Irvin et al., in press) and as the larvae of this species are highly polyphagous, the screening of selective food plants in the laboratory is necessary to reduce the chances of exacerbating *E. postvittana* populations in vineyards where habitat manipulation is employed.

The aim of this study was to screen potential 'selective food plants' which would enhance the fitness of *D. tasmanica* without enhancing that of *E. postvittana.*

2.3 Materials and methods

A laboratory colony of *E. postvittana* was established using eggs obtained from a colony maintained at HortResearch, Mt Albert, New Zealand. Larvae were reared on an artificial diet (Singh, 1983) in plastic containers, 7 cm in diameter and 4.7 cm high, at 20 ± 2 °C with a 16L: 8D photoperiod. Pupae were collected from the plastic containers and placed in plastic bags until adult moths emerged. Any *E. postvittana* eggs that were laid on the bags were removed and used to rear subsequent generations.

A colony of *D. tasmanica* was established from insects reared from leafroller larvae collected in vineyards in Marlborough and Canterbury, New Zealand. *D. tasmanica* was reared on *E. postvittana* larvae maintained at 20 ± 2 °C with a 16L: 8D photoperiod based on methods adapted from Berndt (2002).

Plants of buckwheat, mustard, bishop's flower and dill were grown from seeds in a glasshouse at Lincoln University and were used in the experiments when all plants were flowering simultaneously. Buckwheat was chosen as in a previous study it was found to enhance the longevity of *D. tasmanica* (Irvin et al., in press). The other three species were chosen as they were easily available as seed, they had fast sowing to flowering times and they were being used in a flowering seed mix sold by Kings Seed Co. in New Zealand to attract beneficial insects.

2.3.1 *E. postvittana* fitness

Six replicates of each of six treatments (no food and no water, water only, buckwheat, mustard, bishop's flower and dill) were set up in a complete randomised block design at 20 ± 2 °C and with a 16L: 8D photoperiod. In the flowering plant treatments a flowering shoot still connected to a potted plant was inserted into the base of a cylindrical cage (20 cm long and 9 cm diameter) made of clear acetate sheeting. The top of the cage was covered in a fine nylon mesh and the base of the cage sealed with a foam plug with a 4 cm long cut in the foam into which the flowering shoot could be inserted. A small hole was made in the side of the cage to allow insects to be introduced and removed from the cage and this was sealed with a plug of cotton wool. In the 'no food and no water' and 'water only' treatments, the base of the cage was replaced with a clear plastic Petri dish. A cotton wool wick (2 cm long) that had been soaked in water was placed in all treatments (except the 'no food and no water' treatment) to provide water. The cotton wool wick was re-soaked in water every two days and plants were replaced as required.

A pair of newly emerged adult *E. postvittana* from the laboratory colony was placed in each treatment. Cages were checked daily for adult moth survival and every 3-4 days the cages were removed and replaced and the number of eggs laid on the cages and on the plants was counted using a stereo microscope. Survival rates and lifetime fecundity of adult *E. postvittana* were compared between treatments using an analysis of variance (ANOVA) with a randomised block design. Significant effects were further explored using a Fisher's Least Significant Difference (LSD) test.

2.3.2 *D. tasmanica* fitness

Longevity

Newly emerged male and female *D. tasmanica* from the laboratory colony were randomly allocated to one of five replicates of each of the six treatments. Individual *D. tasmanica* were released separately into the cages and these were checked daily for parasitoid survival. The longevity of *D. tasmanica* was compared between treatments using an ANOVA and a Fisher's LSD test was used to compare differences between the treatments.

Potential fecundity

As buckwheat was the only flowering plant species to increase the longevity of *D. tasmanica* and there is no evidence in the literature to suggest that parasitoid fecundity may be increased by sugar feeding without increasing parasitoid longevity, buckwheat was the only species tested for its effects on *D. tasmanica* fecundity. Newly emerged *D. tasmanica* from the colony were randomly assigned to one of seven treatments. These were: emergence, 12 hours unfed, 24 hours unfed, 48 hours unfed and 12, 24 and 48 hours exposed to buckwheat flowers. The parasitoids

assigned to the 'emergence' treatment were killed within two hours of emergence. Those in the unfed treatments were placed in individual cages (as described above) and were provided with a cotton wick soaked in water. *D. tasmanica* which were exposed to buckwheat were placed in a cage with a shoot with flowers (as described above) and water via a water soaked cotton wick. *D. tasmanica* was maintained in these cages for 12, 24 or 48 hours, depending on the treatment to which they were assigned. After the allocated time, *D. tasmanica* individuals were transferred to individual microcentrifuge tubes and were placed in a freezer. After being in the freezer for at least 24 hours, parasitoids were dissected on a microscope slide by carefully pulling the parasitoid's ovipositor from its abdomen with fine forceps, exposing the ovaries. These were then stained with a 0.1 % methyl blue solution and crushed with a cover slip. The number of eggs in each parasitoid was counted under a stereo microscope (40 x magnification). Ten replicates of each treatment were conducted at 20 ± 2 °C and a 16L: 8D photoperiod. The potential fecundity of *D. tasmanica* was analysed over time and between the treatments using a generalised linear model with a Poisson distribution.

2.3.3 *E. postvittana* larval feeding preference

Fifty Petri dishes (6 cm diameter) were fitted with a piece of damp filter paper on the bottom of each dish and a piece of dry filter paper into the top of each dish. Leaves (less than 3 cm in diameter) of buckwheat, bishop's flower, dill, mustard and grapevine (cv. Sauvignon Blanc) were randomly arranged around the perimeter of the dish. Except for the grapevine leaves, all of the leaves were collected from potted plants grown in a glasshouse at Lincoln University to which no insecticides had been applied. Grapevine leaves were collected from a vineyard block at Lincoln University.

One first-instar *E. postvittana* larva from the laboratory colony was placed in the centre of each dish which was sealed with Parafilm to prevent the larvae escaping. The larvae were left in the dishes for 24 hours after which time the dishes were opened and the leaf on which each larva was feeding or positioned under or on was recorded. This was considered to be the "preferred" plant species. The preference of first-instar *E. postvittana* larvae for the different leaves was compared using a generalised linear regression model, with binomial proportions, where differences between the treatments were compared using Student's t-tests.

2.4 Results

2.4.1 *E. postvittana* fitness

There were no significant differences in the longevity of adult female *E. postvittana* between the six treatments ($F = 1.92$, $df = 6$, $P = 0.116$; Fig. 2.1a). However, adult male longevity was affected ($F = 4.84$, $df = 6$, $P = 0.003$; Fig. 2.1b); the survival of the adult male moths was significantly lower in the no food and no water treatment (Table 2.1). Compared with the water only treatment, the longevity of both female and male adult *E. postvittana* were not significantly affected by the flowering plant treatments (Table 2.1). Also, the lifetime fecundity was not significantly affected by the treatments ($F = 0.53$, $df = 6$, $P = 0.784$; Table 2.1), although the lowest number of eggs laid was recorded in the no food and no water treatment. The majority of eggs laid by *E. postvittana* were on the cages and therefore it was thought that not having a plant stimulus in the control treatments did not affect the result.

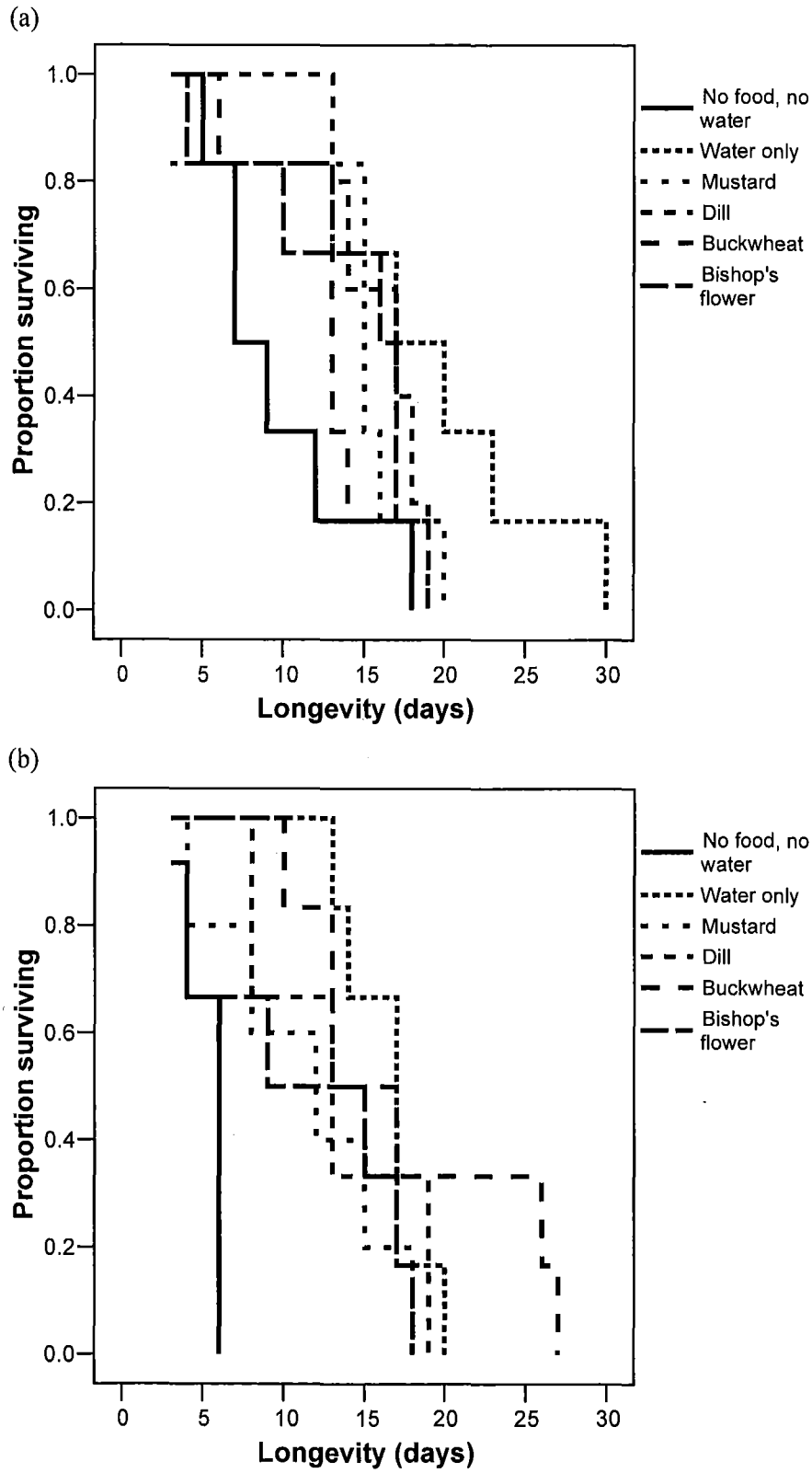

Fig. 2.1 Survivorship curves for adult female (a) and male (b) *E. postvittana* in different treatments.

Table 2.1 Mean (± SE) longevity of adult female and male *E. postvittana* in the different treatments and the mean (± SE) number of eggs laid in each treatment. Significant differences between treatments are indicated using different letters ($P < 0.05$). Where there were no significant differences between treatments, letters are not used.

Treatment	Female longevity (days)	Male longevity (days)	Number of eggs laid
No food and no water	9.7 ± 1.9	5.2 ± 0.5 a	145.0 ± 47.7
Water only	17.8 ± 3.6	16.3 ± 1.0 bc	282.3 ± 117.0
Buckwheat	13.0 ± 1.7	17.7 ± 2.9 c	284.2 ± 84.0
Mustard	12.0 ± 2.9	12.3 ± 2.5 bc	297.0 ± 97.5
Bishop's flower	13.7 ± 2.3	11.0 ± 2.7 ab	313.3 ± 117.5
Dill	16.0 ± 1.3	13.3 ± 3.4 bc	410.8 ± 118.4

2.4.2 *D. tasmanica* fitness

Longevity

The longevity of female *D. tasmanica* was significantly increased by the provision of buckwheat ($F = 7.64$, $df = 5$, $P < 0.001$; Fig. 2.2a) but was not affected by the other treatments (Table 2.2). Male longevity was also significantly different between the treatments ($F = 2.72$, $df = 5$, $P = 0.044$; Fig. 2.2b), where buckwheat significantly increased it compared with the other treatments (Table 2.2).

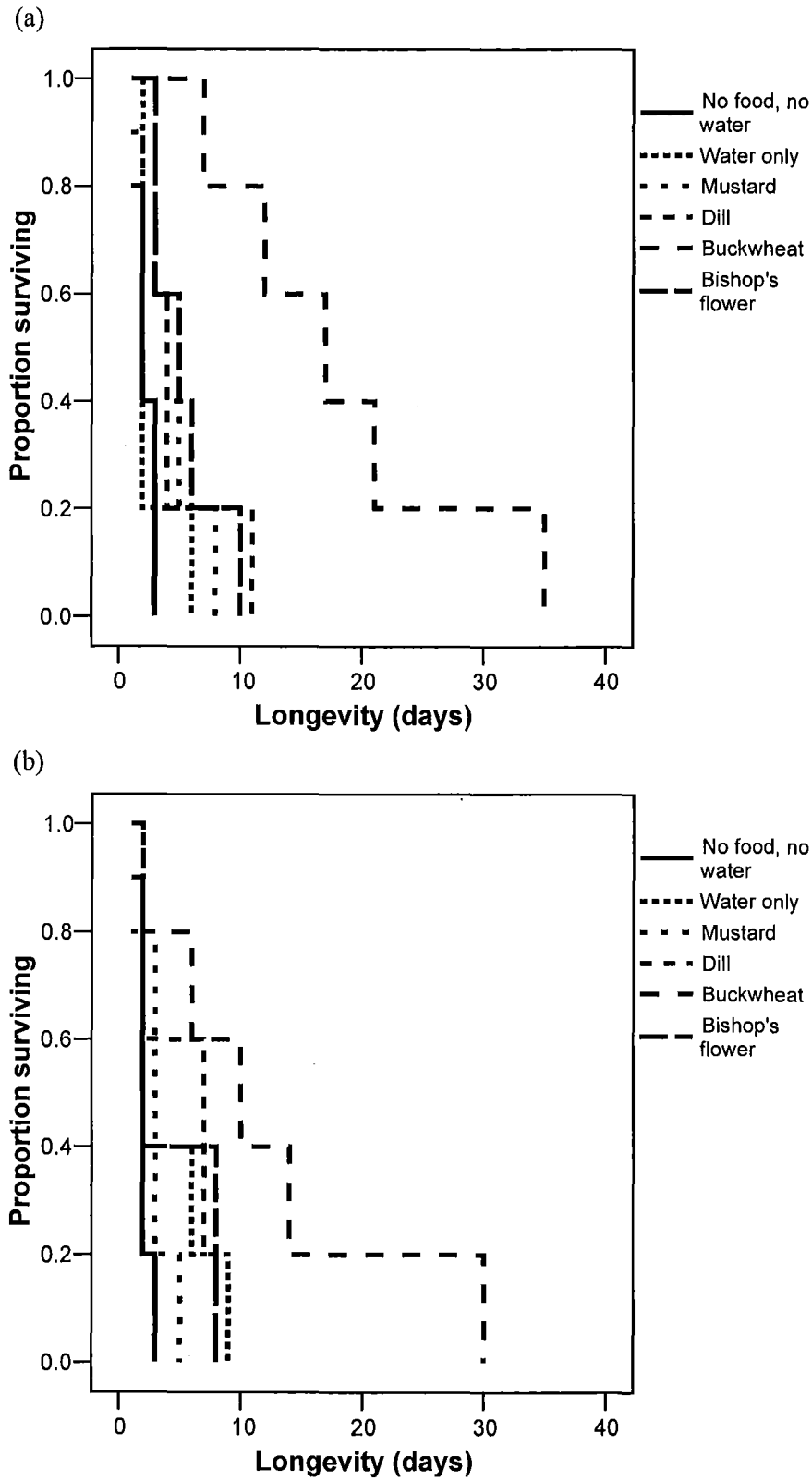

Fig. 2.2 Survivorship curves for adult female (a) and male (b) *D. tasmanica* in different treatments.

Table 2.2 Mean (± SE) longevity of adult female and male *D. tasmanica* in the different treatments. Significant differences between treatments are indicated using different letters ($P < 0.001$).

Treatment	Female longevity (days)	Male longevity (days)
No food and no water	2.0 ± 0.4 a	2.0 ± 0.3 a
Water only	2.8 ± 0.8 a	3.8 ± 1.6 a
Buckwheat	18.4 ± 4.8 b	12.4 ± 4.8 b
Mustard	4.0 ± 1.2 a	3.2 ± 0.5 a
Bishop's flower	5.4 ± 1.3 a	4.2 ± 1.6 a
Dill	5.0 ± 1.5 a	5.2 ± 1.3 a

Potential fecundity

Significantly more eggs were found in the ovaries of *D. tasmanica* in the buckwheat compared with the unfed control treatment (df = 1, $P < 0.001$; Fig. 2.3). The number of eggs in both treatments increased significantly from the number at emergence (df = 1, $P < 0.001$; Fig. 2.4), suggesting that *D. tasmanica* does not emerge with its full quantity of eggs but matures eggs over its lifetime.

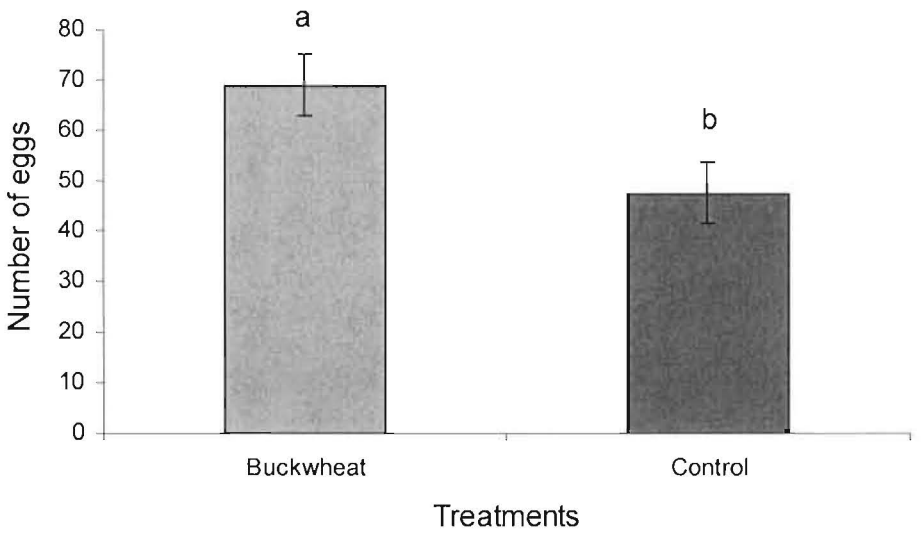

Fig. 2.3 Mean (± SE) number of eggs found in the ovaries of female *D. tasmanica* at 0, 12, 24 and 48 hours in buckwheat compared with unfed control treatments. Significant differences are indicated by different letters ($P < 0.001$).

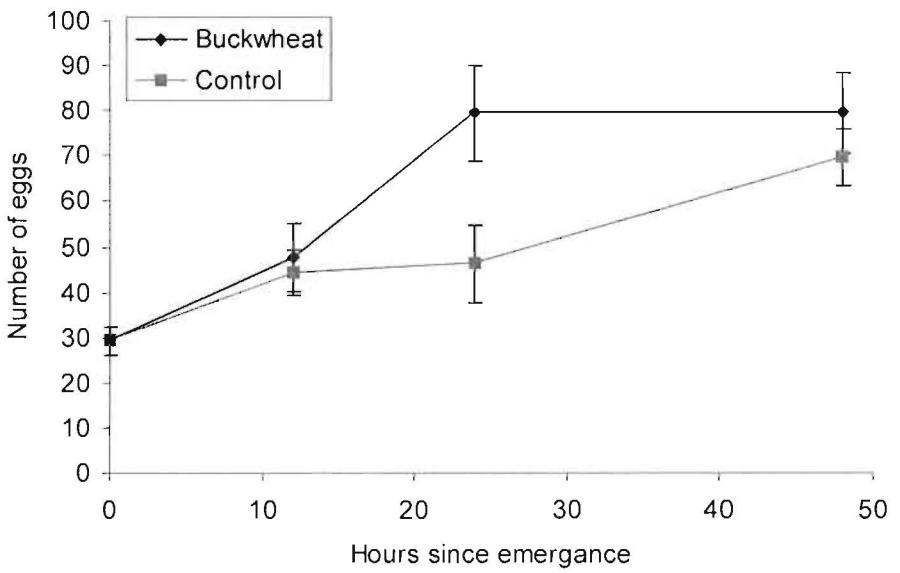

Fig. 2.4 Mean (± SE) number of eggs per female in the dissected ovaries of *D. tasmanica* over time since emergence in buckwheat and unfed control treatments.

2.4.3 *E. postvittana* larval feeding preference

There were significant differences in the plant species preferred by first-instar *E. postvittana* larvae ($F = 14.48$, $df = 4$, $P < 0.001$), with significantly more larvae preferring grapevine leaves to those of the other four plant species (Fig. 2.5). First-instar *E. postvittana* preferred buckwheat leaves to bishop's flower leaves ($t = 0.045$), dill leaves ($t = 0.023$) and mustard leaves ($t = 0.017$). A small percentage of larvae in this experiment either died or after 24 hours had not moved towards any one of the leaves and therefore could not be recorded as having made a choice. The percentages of larvae for which this occurred are not shown in Figure 2.5.

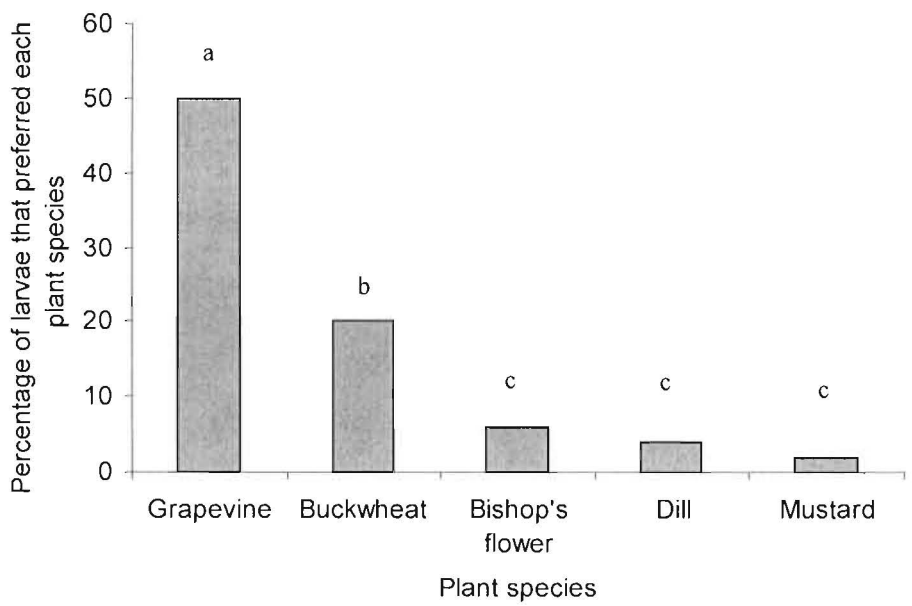

Fig. 2.5 The percentage of first-instar *E. postvittana* larvae that "preferred" each plant species in five-way test arena. Significant differences are indicated by different letters ($P < 0.001$).

2.5 Discussion

2.5.1 Floral resource selectivity

The effects of floral resources on the biological control of *E. postvittana* and other leafroller species has recently been widely studied in vineyards and orchards in Australia and New Zealand (Stephens et al., 1998; Irvin et al., 2000; Berndt et al., 2002; Berndt & Wratten, 2005; Begum et al., in press; Gurr et al., in press; Irvin et al., in press; Scarratt et al., in press). However, there has been criticism in the literature regarding the use of these resources in agricultural systems without a detailed understanding of the effect of these resources on the pest or on higher trophic levels (Stevens et al., 2002). Baggen & Gurr (1998) explained the need to use 'selective food plants' that would increase the fitness of natural enemies without enhancing that of the pest. Therefore, the aim of the present study was to screen flowering plants and to evaluate their potential for use as 'selective floral resources' to enhance biological control of leafrollers in New Zealand vineyards.

2.5.2 *E. postvittana* fitness

In this study, buckwheat, mustard, bishop's flower and dill did not enhance the longevity of adult *E. postvittana.* As most adult Lepidoptera feed on carbohydrate-rich food sources, primarily floral nectars rich in sucrose and amino acids (Romeis & Wäckers, 2000), some effects of nectar feeding were expected. However, access to the nectar of these flowering plant species may have been restricted by corolla depth and proboscis length of *E. postvittana* (Corbet, 2000). Although the provision of floral resources did not influence the longevity of *E. postvittana,* the longevity of males of this species was significantly decreased in the no food and no water treatment, suggesting that a lack of water may have contributed to early mortality in males. This

finding supports the work of Gu and Danthanarayana (1990), in which *E. postvittana* provided with water had a greater longevity compared with those with no food or water. However, these results contradict findings of Begum et al. (in press), who found that when this insect had access to buckwheat flowers, their longevity was significantly increased compared with when they were provided with buckwheat without flowers. Begum et al. (in press) suggested that adult moths were feeding on the buckwheat flowers and consequently had enhanced longevity; however, the effect of buckwheat flowers on the fecundity of *E. postvittana* was not determined. Also, these authors did not provide water to the insects in any of the treatments. Therefore, it is likely that *E. postvittana* was feeding on the buckwheat flowers to access water, in the nectar, resulting in increased longevity in this treatment, whereas in the buckwheat without flowers there was no water available, leading to reduced longevity. In the present study, the fecundity of *E. postvittana* was not influenced by exposure to floral resources, supporting the idea that it did not feed on them. Although not significantly different, *E. postvittana* laid fewer eggs in the no food and no water treatment compared with the other treatments, further supporting the idea that *E. postvittana* may be limited by water. Gu and Danthanarayana (1990) also showed that there were no significant differences in the fecundity of honey fed and water fed *E. postvittana* and they suggested that the reproductive potential of this insect is determined by the availability of water rather than supplemental nutrition at the adult stage.

2.5.3 *D. tasmanica* fitness

Of the four flowering plant species tested, buckwheat was the only one that increased *D. tasmanica* longevity. One reason may be the compatibility of the floral architecture

with the insect's morphology (Jervis et al., 1993; Idris & Grafius, 1995; Patt et al., 1997; Lavandero et al., in press). However, other factors such as diurnal pattern of nectar secretion, nutritional value of nectar and pollen, and floral phenology (Wood, 1961; Baker & Baker, 1983; Wäckers, 2004) may have influenced the fitness of *D. tasmanica.* For example, Azzouz et al. (2004) found that the longevity of *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) was increased when sugar concentrations fed to the parasitoid were increased. Therefore, it is plausible that *D. tasmanica* may have fed on the flowers of all the plant species tested, but only buckwheat nectar had sugar concentrations or ratios that enhanced the fitness of this species.

A greater number of eggs was found in the ovaries of *D. tasmanica* that had been exposed to buckwheat, compared with those fed water only. These results support the work of Irvin et al. (in press), where buckwheat enhanced the potential fecundity of five-day-old *D. tasmanica* by 62%, compared with water only. Also, the number of eggs in both treatments increased from emergence, suggesting that this species is synovigenic (Jervis & Copland, 1996). A synovigenic parasitoid species produces eggs over its lifetime (Jervis & Copland, 1996) and requires proteins, amino acids and lipids to do so. As *D. tasmanica* does not host feed, it must rely on reserves left over from larval stages or from nutrients in nectar to reach their full reproductive potential. As lifetime nutrient allocation to egg production was not analysed here, it is difficult to comment on the probability of floral resources being an essential food for egg production in this species, but as more eggs were produced by those wasps which were exposed to buckwheat compared with those which were provided with water only, it seems likely that floral nectar enhances the fecundity of *D. tasmanica.* Therefore, providing floral resources will not only enhance the longevity of *D.*

tasmanica but may also enhance the potential fecundity of this species, potentially leading to an overall enhancement in biological control of leafrollers in the vineyard system.

2.5.4 *E. postvittana* larval feeding preference

In a multi-choice experiment, first-instar *E. postvittana* larvae chose grapevine leaves over buckwheat, mustard, bishop's flower and dill leaves. However, buckwheat was the second most 'preferred' species by first-instar *E. postvittana* larvae. This result may suggest that buckwheat can be used by leafrollers however, Irvin et al. (in press) showed that *E. postvittana* larvae did not survive as well when fed buckwheat leaves in a laboratory experiment than when they were fed apple leaves. Therefore, buckwheat may be a used as a 'selective food plant' to enhance the biological control of *E. postvittana* and other leafroller species in New Zealand. However, before this is planted in New Zealand vineyards, its effects on populations of leafrollers and their associated natural enemies and the effects of these floral resources on multi-trophic interactions should be considered.

2.5.5 Conclusions

In conclusion, the results of the present study indicate that of the species tested here, buckwheat may be the most appropriate candidate as a 'selective floral resource' to enhance the biological control of leafrollers, specifically *E. postvittana* in New Zealand vineyards. The reasons why buckwheat is such an effective floral resource are unclear, but may be related with the fact that the small flowers are well suited as a nectar source for small hymenopterans, as the nectaries are easily accessible (Lovei et al., 1993) or the fact that buckwheat has a high nectar quality (Vattala, unpublished

data). Further efforts to identify why buckwheat is such an effective 'selective floral resource' would allow CBC researchers to screen other flowering plant species for such selectivity.

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Chapter 3 A pilot study examining the effect of flowering buckwheat on parasitoid abundance and parasitism rates of leafroller larvae in the vineyard

3.1 Introduction

Insect pests tend to be less abundant in more diverse agricultural systems (Risch et al., 1983; Andow, 1991; Thies & Tscharntke, 1999). Root (1973) proposed two hypotheses to explain this. The "resource concentration" hypothesis suggests that insect pests are more likely to find and remain on host plants that are growing in dense or pure stands. As both visual and chemical stimuli from the host and non host-plants affect the rate at which herbivores colonise habitats and their relative behaviour in those habitats, it is reasoned that these cues are stronger from monocultures. The "enemies" hypothesis proposes that fewer insect pests exist in polycultures due to increases in natural enemy populations, which are more effective in these environments. Natural enemies are thought to be more effective in more diverse systems as they are provided with resources which are previously absent or scarce, such as plant-based foods (nectar, pollen and/ or homopteran honeydew), shelter and/ or alternative hosts or prey (Landis et al., 2000; Gurr et al., 2004).

Leafrollers, which are key pests of grapevines in New Zealand (Baker et al., 1994), are the pest insects being studied here. They are attacked by a wide range of natural enemies throughout most of their developmental stages (Thomas, 1989). Of these natural enemies, parasitoids which effect the highest parasitism rates include *D. tasmanica, G. demeter* and *Glab. stokesii.* Of these species *D. tasmanica* is the most common parasitoid in New Zealand (Charles et al., 1996; Suckling et al., 1998;

Berndt, 2002) and it causes the highest parasitism rates of leafroller larvae in New Zealand (Thomas, 1989; Berndt et al., 2002; Berndt et al., in press; Irvin et al., in press).

Therefore, the aims of this study were to examine the effects of flowering buckwheat on the abundance of *D. tasmanica, G. demeter* and *Glab. stokesii* in vineyards and to examine parasitism rates of leafrollers by *D. tasmanica* in the presence of flowering buckwheat.

When this project started, it was thought that there were a number of distinct species within the genus *Dolichogenidea*, of which *D. tasmanica* was one and that a revision of the genus was necessary (J. Berry, pers. comm.). During this study, a visit was made to J. Berry, HortResearch, Auckland, and morphological differences between the species were noted. Therefore, prior to the visit, *Dolichogenidea* spp. collected from the field were treated as one taxon and after learning how to differentiate between the species, *D. tasmanica* was used.

3.2 Materials and methods

3.2.1 Site description

Experiments were conducted in a vineyard block (cv. Chardonnay) at Seresin Estate, Marlborough, New Zealand. The vineyard block was 2 ha and consisted of 110 rows, which were approximately 80 m in length. The vineyard was managed organically and therefore no insecticides were applied. In the block, eight replicates of two treatments (buckwheat and control) were established. Buckwheat (cv. Katowase) was sown into cultivated ground at 45kg/ha on 23 November and 21 December 2003.

Buckwheat was sown in a one and a half meter wide strip, the complete length of the inter-vine row between rows 9-10, 21-22, 33-34, 45-46, 57-58, 69-70, 81-82, 93-94 and flowered from 6 January until 28 April 2003. In March 2003 the upper two thirds of the plants in the first sowing of buckwheat were removed to encourage lateral growth and to prolong flowering via growth from axillary buds. Control rows, which consisted mainly of grasses, clover and weeds, were positioned between rows 3-4, 15-16, 27-28, 39-40, 51-52, 63-64, 75-76, 87-88. Therefore, the distance between each replicate was five rows (approximately 10 m) and the treatments alternated across the vineyard block and were not randomly located. The reason that this experiment was not established as a complete randomised block design was that the vineyard block was sown with buckwheat prior to the start of this project. Also, the control treatments were not sown with flowering plants as Irvin (1999) demonstrated that there were no differences in the number of parasitoids caught in buckwheat areas with flowers and buckwheat areas with flowers removed. Therefore, Irvin (1999) believed that it was the flowers that were attracting and retaining parasitoids and not differences in microclimate.

3.2.2 Pest abundance: pheromone traps

To determine the abundance of adult *E. postvittana*, two pheromone traps were placed in the block, one at the southwestern end and one at the northeastern end. The traps were hung from the fruiting wire and were positioned approx. 1 m from the ground. Traps were placed in the vineyard from 21 January 2003 and were collected fortnightly. After April 2003 few moths were being caught in the traps, so traps were checked monthly. On each of these collection dates, the sticky bases on which the moths were caught were changed and the number of adult male *E. postvittana* was

counted in each trap. Pheromone plugs emitting pheromones to attract male *E. postvittana* (Newcomb et al., 2002) (obtained from Fruitfed Supplies Ltd., New Zealand) were replaced every six weeks. The average number of adult male *E. postvittana* collected on both traps was calculated per trap per day.

3.2.3 Parasitoid abundance: yellow sticky traps

To determine whether flowering buckwheat had an effect on parasitoid abundance, four yellow sticky traps (Trappit, Agrisense-BCS-Ltd., U.K., sourced from Fruitfed Supplies Ltd., New Zealand) (24 cm x 20 cm) were placed in the middle of each buckwheat and control replicate. Traps were hung from the fruiting wire and were positioned 1 m from the ground. Traps were placed in the vineyard before the buckwheat began to flower on the 19 December 2002 and they were maintained in the vineyard for two weeks and after this time they were removed and replaced. After being removed, the traps were covered in clear plastic cling wrap and were transported back to Lincoln University where parasitoids were identified. This procedure was repeated until the end of April 2003.

The average number of each parasitiod species was analysed over time and any differences in the number of parasitoids caught on traps in buckwheat and control treatments was compared using a repeated measures ANOVA (Genstat, version 7.0, 2003).

3.2.4 Parasitoid abundance: suction samples

In a separate experiment, a motorised suction sampling machine (Arnold, 1994) was used to collect insects from five flowering plant species; buckwheat, alyssum cv.

Carpet of Snow, coriander, *Coriandrum sativum* (L.) (Apiaceae), phacelia, *Phacelia tanacetifolia* Benth. (Hydrophyllaceae) and mustard, *Brassica chinensis* L. (Brassicaceae) cv. Choi Sum. These species were each planted in 5 m by 0.5 m areas, with a 2 m gap between each species, replicated five times along a 185 m vine row in a complete randomised block design in a vineyard block (cv. Sauvignon Blanc) at Seresin Estate. Buckwheat, coriander, phacelia and mustard seeds were sown by hand on the 18 December and then again on the 18 February. Alyssum plants were purchased as mature plants and were planted on the dates above. This successive planting permitted flowering throughout the entire length of the project.

Suction samples were taken from these plants three times throughout the season; 17 March 2003, 17 April 2003 and 29 April 2003. Samples were taken at the height of flowers to capture insects which were feeding on them. Each suction sample was taken for 50 s along each 5 m flowering plant section. Samples were removed from the suction sampling machine and were placed in a container with ice, to reduce insect activity.

Insects were stored in a freezer (-50 °C) until they could be identified. The number of *Dolichogenidea* spp. collected was recorded for each sample taken. The mean number of *Dolichogenidea* spp. collected from each plant species was calculated and a "preference" for each plant species was determined.

3.2.5 Parasitism rates of naturally-occurring leafroller larvae

Prior to searching for leafroller larvae on vines, timed searches were conducted to optimise searching efficiency. Ten randomly selected vines were searched in a block

of vines (cv. Sauvignon Blanc) and each was searched for eight minutes. After each minute, the number of leafroller larvae found was recorded. The cumulative number of larvae collected after each minute of vine searching was plotted (Fig. 3.1). Six minutes was selected as the optimum. Therefore, in all further experiments, six minutes was the standard search time.

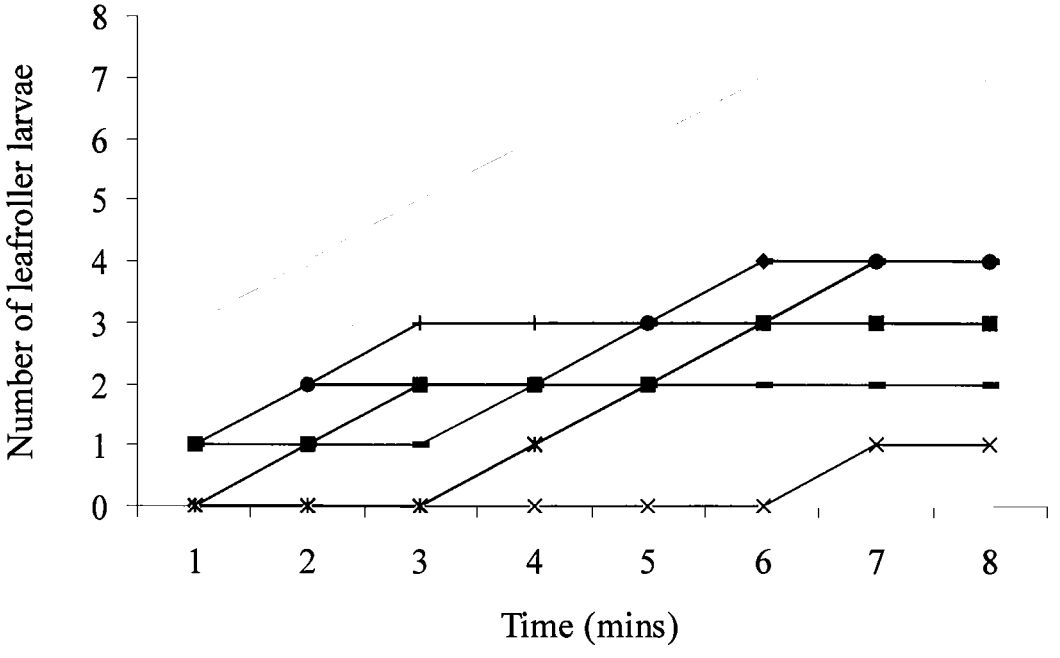

Fig. 3.1 The cumulative number of leafroller larvae collected from timed searches of vines. Each line represents a different vine searched.

On 19 February, 19 March and 18 April 2003, four vines were randomly selected and searched for six minutes for leafroller larvae in each buckwheat and control row. Any larvae found were placed into a tube containing diet (Singh, 1983). Diet tubes were labelled with the date and location of collection and were transported back to Lincoln University where they were placed in a temperature-controlled room at 20 °C ± 2 °C

and a 16L: 8D photoperiod until a parasitoid emerged or until the larvae became adult moths. Parasitoids were identified to species where possible.

The percentage of leafroller larvae parasitised by *D. tasmanica* in each treatment was compared on each collection date separately and then again when all three dates were pooled using a generalised linear regression model with binomial proportions (Genstat, version 7.0, 2003). Parasitism by species other than *D. tasmanica* was not considered as previous studies have shown that parasitism of leafroller larvae by *D. tasmanica* is over 85% of total parasitism and that parasitism by *Glyptapanteles* spp. and *Glab. stokesii* comprised of less than 5% parasitism (Charles et al., 1996; Berndt et al., in press).

3.2.6 Parasitism rates of released leafroller larvae

Leafrollers were released in the event that naturally-occurring populations remained low. Batches of 100 *E. postvittana* eggs, which had been laid on a piece of wax paper, were placed out in the vineyard block on 23 March and 16 April 2003. Egg batches were stapled to the upper side of a vine leaf on two randomly selected vines in the centre of each buckwheat and control replicate. Vines where *E. postvittana* eggs were released were tagged with flagging tape so that release sites could be easily identified. Leaves, to which an egg batch had been stapled, were covered with calico bags (Irvin et al., in press) to allow the eggs to hatch without being predated upon. The bags were removed 5-7 days later when first-instar larvae began to hatch. Two weeks after the bags had been removed, the release sites were searched for *E. postvittana* larvae. Any larvae found were collected and placed in diet tubes (Singh, 1983) and were reared in

a temperature-controlled room at Lincoln University under the conditions described above.

The percentage of leafroller larvae parasitised in each treatment was compared on each collection date separately and then again when all three dates were pooled, using a generalised linear regression model with binomial proportions (Genstat, version 7.0, 2003).

3.2.7 Sugar feeding by *D. tasmanica*

To compare sugar feeding in buckwheat and control treatments, parasitoids were collected using a suction sampling machine. Suction samples were collected on two sampling dates, 18 March and 17 April, for one minute and over an area of approximately $2m^2$. Two samples were taken just above the buckwheat flowers in the buckwheat replicates and just above the mowed grass in the control replicates. Samples were placed in the freezer (-50°C) and were transported back to Lincoln University for parasitoid identification. Any *D. tasmanica* that were collected were analysed for fructose presence using the cold anthrone test (Walsh & Garms, 1980; Stewart & Kline, 1999). This test involved placing *D. tasmanica* in a 1.5 ml micro centrifuge tube, adding 200 µl of anthrone reagent to each tube and crushing the *D. tasmanica* with a plastic pestle. The pestle was rinsed between specimens in a beaker of water and then dried with a paper towel. One centrifuge tube was used as a blank after testing four *D. tasmanica* for fructose presence. The blank tube contained 200 µl of anthrone reagent and the plastic pestle was placed in the tube to ensure that sugars were not being transferred between tubes. The tubes were checked after one hour and if a colour reaction occurred (anthrone solution changed from yellow to green), then it

was deemed that fructose was present in the body of *D. tasmanica* and it was considered to be sugar fed.

3.3 Results

3.3.1 Pest abundance: pheromone traps

The highest number of male *E. postvittana* collected in pheromone traps was on 1 April, when ten adult moths were caught per day in each trap (Fig. 3.2). After April, the number of moths caught decreased until July when very few were caught.

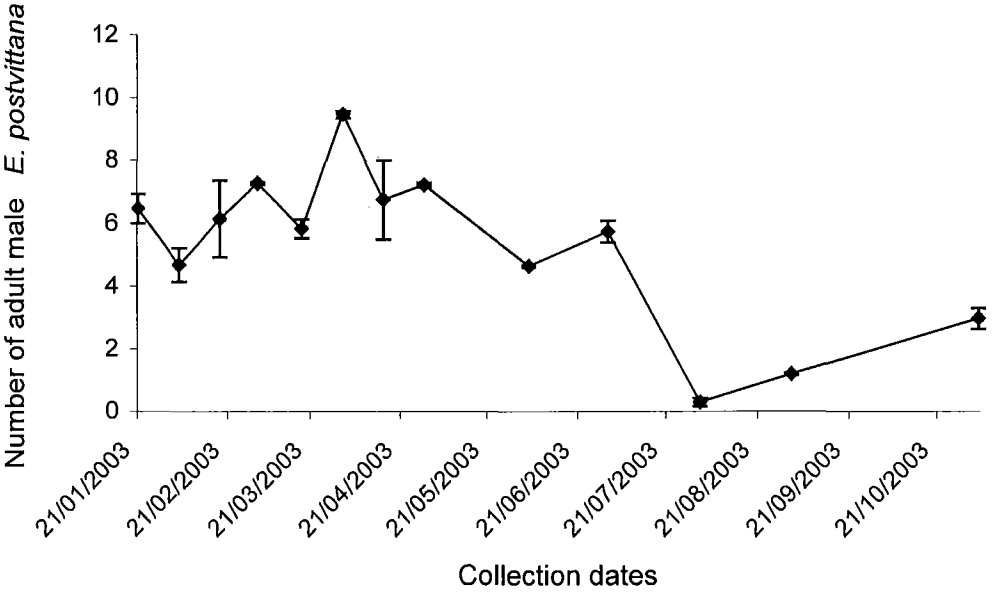

Fig. 3.2 Mean (± SE) number of male *E. postvittana* collected per pheromone trap per day in 2003.

3.3.2 Parasitoid abundance; yellow sticky traps

There were no significant differences in the number of *D. tasmanica* caught on sticky traps in the buckwheat compared with the control treatments ($F = 2.51$, $df = 1$, $P = 0.136$; Fig. 3.3a). Although a least significant difference test indicated that on the 30

February 2003 there were significantly more *D. tasmanica* caught on sticky traps in the buckwheat rows.

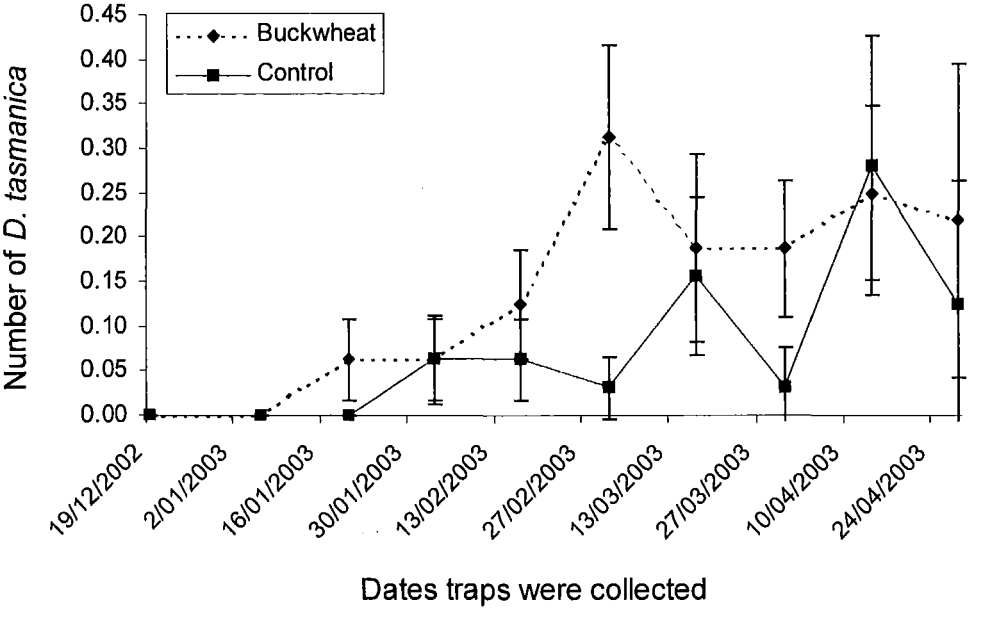

Fig. 3.3a Mean (± SE) number of *D. tasmanica* collected per sticky trap in buckwheat and control treatments over a two-week period.

The number of *Glyptapanteles* spp. collected on traps was significantly higher in buckwheat areas for all trap collection dates ($F = 7.71$, $df = 1$, $P = 0.015$; Fig. 3.3b). There was a significant effect of time on the number of *Glyptapanteles* spp. captured ($F = 8.44$, $df = 1$, $P < 0.001$).

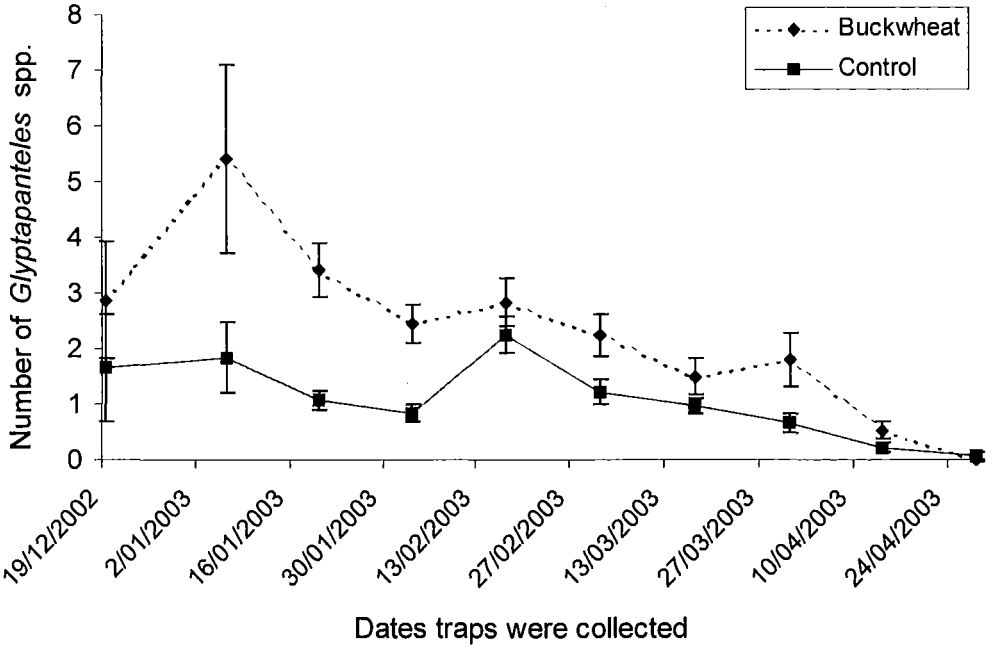

Fig. 3.3b Mean (± SE) number of *Glyptapanteles* spp. collected per sticky trap in buckwheat and control treatments over a two-week period.

Few *Glab. stokesii* were collected on traps throughout the field season; however there was a significant difference in the numbers of this species caught on sticky traps between the two treatments ($F = 6.25$, $df = 1$, $P = 0.025$; Fig. 3.3c), with a higher number caught in the buckwheat treatment. There was also a significant effect of time on the number of this species caught ($F = 9.35$, $df = 1$, $P < 0.001$; Fig. 3.3c).

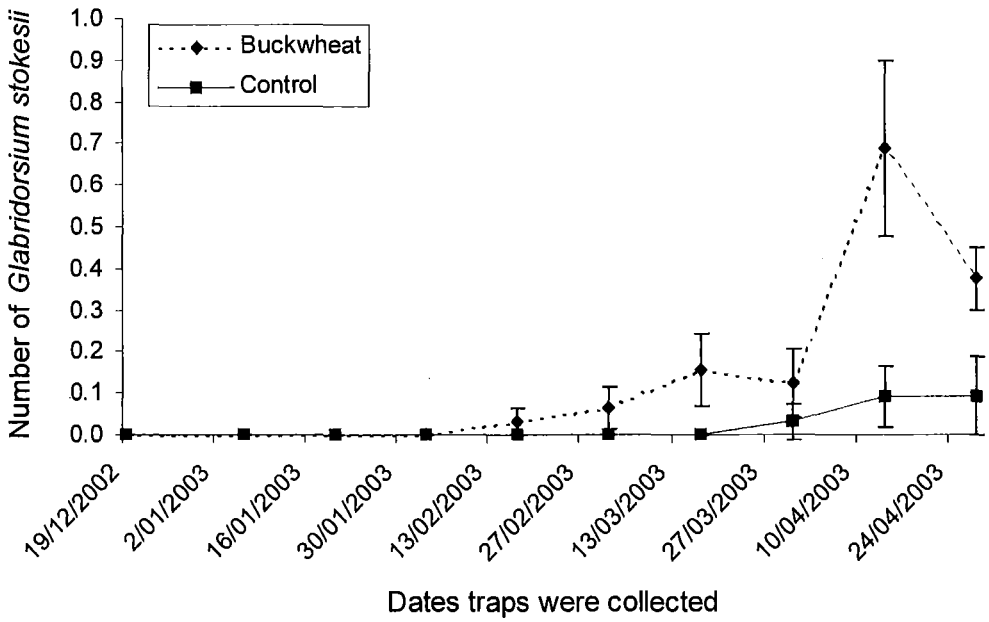

Fig. 3.3c Mean (± SE) number of *Glabridorsum stokesii* collected per sticky trap in buckwheat and control treatments over a two-week period.

3.3.3 Parasitoid abundance; suction samples

The greatest number of *Dolichogenidea* spp. was collected in suction samples from buckwheat flowers (Fig 3.4). After buckwheat, the greatest number was collected from alyssum flowers.

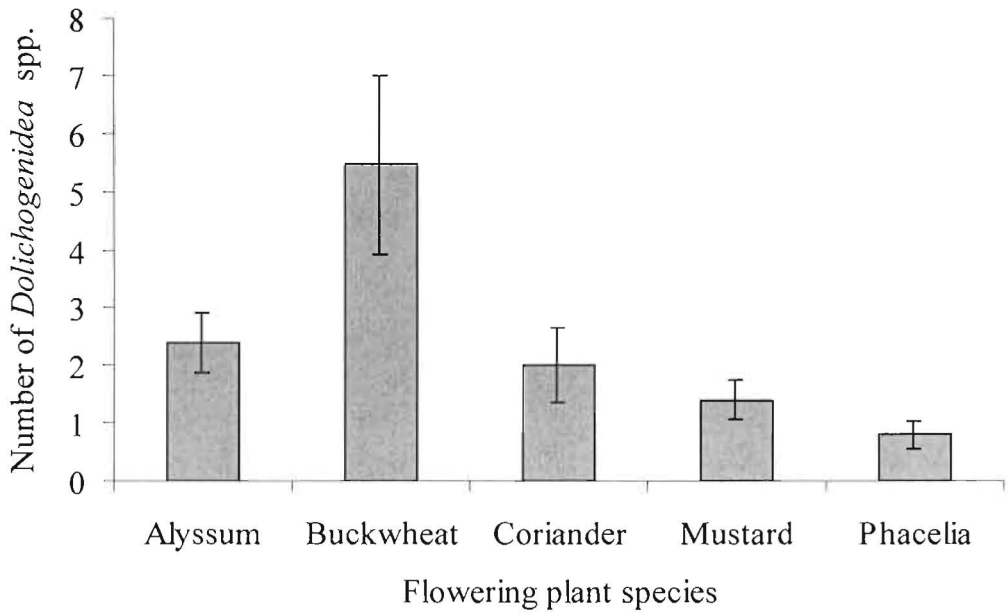

Fig. 3.4 Mean (± SE) number of *Dolichogenidea* spp. collected in suction samples from each of the flowering plant species.

3.3.4 Parasitism rates of naturally-occurring leafroller larvae

Of a total of 68 naturally-occurring leafroller larvae collected on vines in the buckwheat treatment, 57% were parasitised by *D. tasmanica*. Of the 38 larvae collected in the control treatment, only 21% were parasitised by *D. tasmanica*. There was a significant difference between these treatments ($F = 6.92$, $df = 1$, $P = 0.009$; Fig. 3.5).

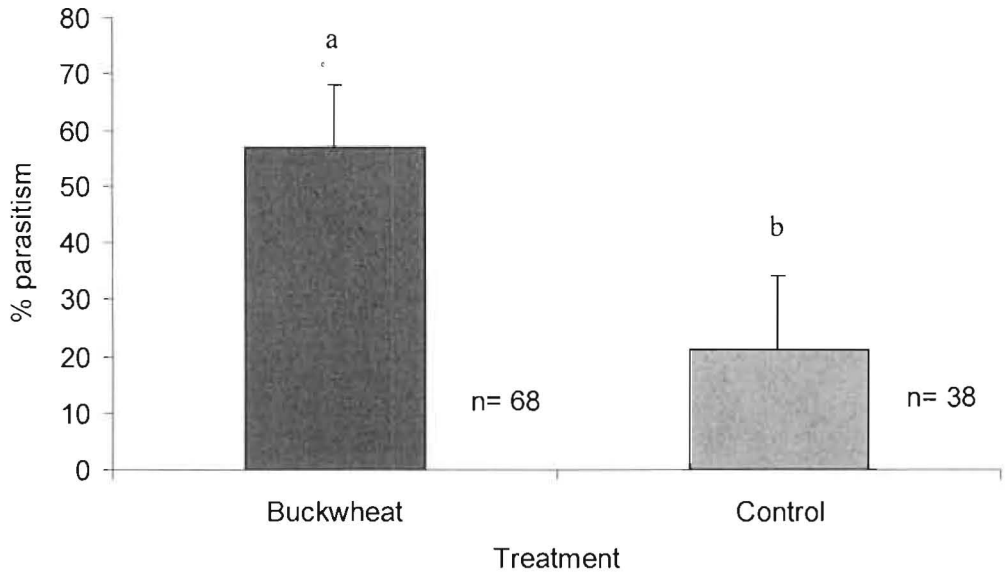

Fig. 3.5 Mean (± SE) number of naturally-occurring leafroller larvae parasitised by *D. tasmanica* in each treatment. Different letters indicate significant differences between the treatments.

3.3.5 Parasitism rates of released leafroller larvae

When percent parasitism of released leafroller larvae was compared in buckwheat and control treatments, there were no significant differences between the two treatments ($F = 0.32$, $df = 1$, $P = 0.574$; Fig. 3.6), with 59% and 49% parasitised in buckwheat and control treatments, respectively.

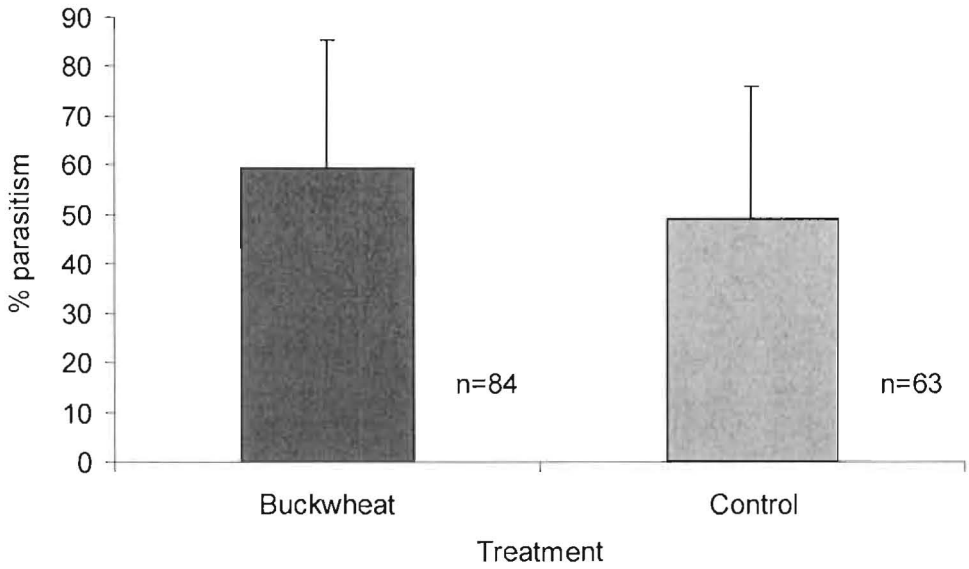

Fig. 3.6 Mean (± SE) number of released-recovered leafroller larvae parasitised by *D. tasmanica* in each treatment.

3.3.6 Sugar feeding by *D. tasmanica*

In the 64 suction samples taken in buckwheat and control treatments, only nine *D. tasmanica* were collected and all of these came from the buckwheat treatment. Seven had fructose in their bodies and two had a negative response to the cold anthrone test.

3.4 Discussion

3.4.1 Leafroller abundance

Leafrollers have three generations per year in Marlborough, New Zealand (Wearing et al., 1991). Adults emerge from over-wintering larvae in October, the second generation of adults occurs in late January and the final generation in late March (Thomas, 1984). In this study, the pheromone traps provided an indication of when the third generation of adult *E. postvittana* appeared in the vineyard in 2003 with peak numbers of adult male *E. postvittana* being recorded in late March to early April. The appearance of the first and second generation of adult *E. postvittana* was not recorded, as traps were not deployed late January.

3.4.2 Parasitoid abundance

When the abundance of three parasitoid species was compared in buckwheat and control areas, significantly more *Glyptapanteles* spp. and *Glab. stokesii* were found in buckwheat than in control areas. Also, more *D. tasmanica* were found in buckwheat than in control areas, but this result was significant on one date only. One possible reason why more *D. tasmanica* were not always found in areas where buckwheat was planted may be because *D. tasmanica* may disperse more widely than 10 m (the distance between the two treatments. Also, in this experiment, yellow traps were used to attract parasitoids, the colour providing a visual stimulus similar to that of a yellow flower. It is likely that parasitoids, which have recently fed on nectar from buckwheat flowers, would be less likely to be attracted to a yellow trap (Takasu & Lewis, 1995). Therefore, different internal hunger states may lead to different trap catches and this needs to be further explored. One method that could be used to explore this would be

the use of different coloured traps or clear traps to determine whether trap colour affected trap catch.

When Irvin (1999) compared the abundance of *D. tasmanica* in areas of an orchard where buckwheat was present and where it was not, more *D. tasmanica* were collected on yellow sticky traps near buckwheat. However, Berndt et al. (2002) did not find more *D. tasmanica* on yellow sticky traps in vineyards planted with buckwheat and they suggested that this may have been due to the low number of parasitoids trapped.

The results from the suction sampling indicated that of the five flowering plant species tested in the vineyard, more *Dolichogenidea* spp. were collected from buckwheat flowers than from any of the other species. Buckwheat is often used in conservation biological control studies to enhance natural enemies (Platt et al., 1999; English-Loeb et al., 2003; Tylianakis et al., 2004; Lee & Heimpel, 2005) as it has a wide and shallow corolla (Lavandero et al., in press), allowing the parasitoids to easily obtain the nectar. Also, buckwheat is useful as a cover crop as it grows and flowers quickly (Bowie et al., 1995) and seed is easily and inexpensively obtainable in New Zealand.

3.4.3 Parasitism rates

Marino & Landis (1996) and Thies & Tscharntke (1999) showed that parasitism rates were higher in more structurally complex landscapes. In this study, more naturally-occurring leafroller larvae were both collected and parasitised in buckwheat compared with control areas of the vineyard. Exactly why there were more larvae collected in

buckwheat compared with control areas is unknown, however, in Chapter 5, the number of larvae collected in the two treatments does not differ and therefore this result was not repeated. Parasitism rates were greater in buckwheat than control areas and whether this occurred as the result of a density-dependent relationship could be further explored. However, parasitism rates of released larvae were not significantly different between the two treatments. This method of releasing larvae was used as a contingency in case naturally-occurring larvae were not abundant. However, Irvin et al. (in press) and Berndt et al. (2002) considered that this method is not the most reliable predictor of parasitism rates as releasing high-density patches of hosts results in increased rates of parasitism in both treatments (density-dependant parasitism is discussed further in Chapter 5).

3.4.4 Sugar feeding

Even though few parasitoids were collected in this experiment, of the nine obtained, seven had fructose in their bodies. Parasitoids which did not test positively for fructose may either have not fed or may have digested their sugar meal. Therefore, the cold anthrone test can provide information only on the number of parasitoids that have recently fed on a sugar meal (within the last 24 hours). Other limitations of this test are that it does not provide information on where the parasitoids obtained their sugar meals. As the vineyard in which this study was conducted is organically managed, there was an abundance of flowering weeds, so any of these species could have provided a sugar source. Sugar analyses can distinguish between sugar types and therefore potentially determine where a parasitoid has fed (i.e., on which flowering plant or on aphid honeydew) (Wäckers & Steppuhn, 2003). Although the actual source of the sugar meal could not be identified in this study, it is most likely that

sugar feeding was occurring on the buckwheat flowers as the samples were taken directly from the flowers and there were no obvious honeydew deposits in the vineyard.

In summary, this study showed that *D. tasmanica* fed on sugar sources in the vineyard (probably on buckwheat flowers), parasitoid abundance was increased near flowering buckwheat and parasitism rates of leafroller larvae by *D. tasmanica* were higher in areas of the vineyard where buckwheat was planted. These results are likely to be due to the enhancement of parasitoid fitness in the field but they may also be due to enhanced microclimate, increased water intake by parasitoids and/or the presence of alternative hosts (Heimpel & Jervis, 2005). However, field and laboratory evidence to date strongly suggests that nectar provisioning is the main factor involved (Heimpel & Jervis, 2005). Together these results indicate that floral resources, such as flowering buckwheat, increase the potential impact of *D. tasmanica* on leafrollers in the New Zealand vineyard system. Further work is required, however, to determine whether pest abundance is reduced by this enhanced rate of parasitism.

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Chapter 4 The influence of floral resource subsidies on dispersal and parasitism rates: marking the parasitoid *Dolichogenidea tasmanica* with rubidium chloride [1]

4.1 Abstract

The dispersal of natural enemies from floral resource subsidies is of particular importance in habitat manipulation research, as the distances that they disperse have consequences for the deployment of these resources in cropping systems. *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) is the most common parasitoid to attack leafroller larvae in New Zealand. Its dispersal from flowering buckwheat, *Fagopyrum esculentum* Moench cv. Katowase plants in an organic New Zealand vineyard, and parasitism rates of leafroller larvae by *D. tasmanica* were measured. Three foliar applications of rubidium chloride (RbCl) were made at three-week intervals to a single strip of buckwheat in the centre of each of five vineyard areas. Yellow sticky traps were placed in each area at distances of 0, 4, 10 and 30 m in both directions from the buckwheat to collect adult *D. tasmanica.* Buckwheat leaf samples were taken following the third application of RbCl to ensure that the plants had been marked with the rubidium. Parasitism of leafroller larvae by *D. tasmanica* was measured by conducting timed searches for leafroller larvae on randomly selected vines at 0, 4, 10 and 30 m from the flowering buckwheat, collecting leafroller larvae and rearing them through to either adult moths or parasitoids. Leaves of the sprayed buckwheat had rubidium concentrations above background and these concentrations persisted for at least nine days following the

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application of the RbCl. *D. tasmanica* had fed on the nectar of the buckwheat plants and dispersed at least 30 m from the plants within a seven-day sampling period. The number of rubidium-marked *D. tasmanica* on traps was fewer at 10 m fro the floral resources but otherwise was uniform across the distances. Parasitism rates of leafroller larvae were greater adjacent to the buckwheat (40 %) than at 10 m from it (19 %), but remained at high levels at 30 m from the buckwheat (36 %). This study demonstrates how RbCl can be used to mark parasitoids to measure their dispersal from floral resource subsidies and therefore to inform decisions on the deployment of floral resource subsidies in conservation biological control.

4.2 Introduction

The dispersal of natural enemies from floral resource subsidies (Wratten et al., 2003; Gurr et al., 2005) is of particular importance in habitat manipulation research, as the distances that they disperse have consequences for the deployment of these resources in cropping systems. Natural enemies, specifically parasitoids, use floral resource subsidies in the field (Maingay et al., 1991; Jervis et al., 1993; Baggen & Gurr, 1998; Tooker & Hanks, 2000) and feed on floral nectar, extra-floral nectar and/ or homopteran honeydew (Leius, 1967; Jervis et al., 1993; Jervis et al., 1996). These food sources provide natural enemies with essential nutrients for survival and reproduction and have been found to increase natural enemy fitness in laboratory experiments (Foster & Ruesink, 1984; Heimpel et al., 1997; Johanowicz & Mitchell, 2000; English-Loeb et al., 2003; Berndt & Wratten, 2005; Irvin et al., in press), increase parasitism rates in the field (Foster & Ruesink, 1984; English-Loeb et al., 2003; Tylianakis et al., 2004) and contribute to pest suppression in agro-ecosystems (Gurr et al., 2004).

However, there is little work which has investigated the movement or dispersal of natural enemies from floral resource subsidies into the cropping system, although there are a number of techniques available to measure such movement (Hagler & Jackson, 2001; Lavandero et al., 2004a; 2004b). For example, dyes may be applied by spraying the floral resource area to mark natural enemies (Schellhorn et al., 2004). However, this method does not identify individuals which have been feeding on, for example, the floral nectar. Internal markers or 'self-marking' techniques, such as the detection of specific sugars in the guts of the natural enemies can be used to provide information on the movement of these insects from floral resources (Heimpel et al., 2004). However, there are limitations in applying this method; sugars may degrade over the life of the insect and therefore may be detectable only for a finite length of time following sugar feeding. Also, the trapping method used to collect the natural enemies must be one which allows analysis of sugars before they degrade.

Since Berry et al. (1972) suggested using rubidium to mark insects, trace elements have increasingly been used to mark arthropods to study their movement in agro-ecosystems (Graham et al., 1978; Jackson et al., 1988; Hopper & Woolson, 1991; Prasifka et al., 2001; Pickett et al., 2004). Commonly, rubidium has been used in mark-recapture studies from overwintering refuges into adjacent crops (Corbett et al., 1996; Pickett et al., 2004) and intercrop movement (Prafiska et al., 2001). However, this element may also be used to self-mark natural enemies, as it readily replaces potassium in biological tissue and therefore is taken up into the nectar and pollen after being watered into the soil or applied as a foliar application (van Steenwyk, 1991; Freeman-Long et al., 1998; Gu et al., 2001). The natural enemies feeding on these

plant parts may then become labelled with rubidium by taking it up in concentrations higher than background levels, making a labelled insect easily detectable. Freeman-Long et al. (1998) used rubidium to study the movement of natural enemies from flowering plants to nearby crops. They applied foliar applications or injected rubidium chloride (RbCl) on or into flowering plants and measured the movement of natural enemies from them. They found that natural enemies were feeding on the plants (either on the pollen or nectar) and were then moving into the associated crops. They also found that many natural enemies were marked at over 30 m from a rubidium-labelled cover crop mix and at 75 m from a rubidium-labelled hedgerow.

Although work has been carried out on the dispersal of *E. postvittana* (Suckling et al., 1994), there is no information on the dispersal of its main parasitoid. Therefore, the aims of this study were to measure the dispersal of the parasitoid *D. tasmanica* from flowering buckwheat to determine how far this species moves after feeding on floral resource subsidies and to measure the effects of floral resources on parasitism rates of leafroller larvae at increasing distances from these resources.

4.3 Materials and methods

4.3.1 Study site

Five areas of grapevines cv. Sauvignon Blanc were selected at Seresin Estate, an organic vineyard in Marlborough, New Zealand for use in this study. The dimensions of the five areas used in the study were 200 m by 90 m, 125 m by 75 m, 180 m by 150 m, 200 m by 95 m and 150 m by 75 m. In each area, buckwheat was sown in a two meter wide strip, the entire length of an inter-vine row in the middle of the area, at a

rate of 45kg/ha on 24 November 2004 and on 22 January 2004 to promote continual flowering throughout the season.

4.3.2 Applications of rubidium chloride

Once the buckwheat plants were flowering, aqueous solutions of rubidium chloride (RbCl) were applied using a 20 L backpack motor-operated sprayer at 1000 ppm (22 February 2004) and at 2000 ppm (14 March and 4 April 2004). Solutions were prepared using solid RbCl (Sigma, Sydney, Australia for the first application and Aldrich APL, Illinois, USA for the second and third applications). The applications were made during early evening, when insect activity was low, to minimise the effect of directly spraying *D. tasmanica* adults. The solution was applied until droplets of the solution were visible on the buckwheat foliage.

4.3.3 Collecting *D. tasmanica* and buckwheat for rubidium analysis

Yellow sticky traps (Trappit, Agrisense-BCS-Ltd., U.K., sourced from Fruitfed Supplies Ltd., New Zealand) (24 cm x 20 cm) were used to collect adult *D. tasmanica.* To determine background levels of rubidium in this insect, 40 traps were placed in a vineyard area distant from the sprayed buckwheat to collect *D. tasmanica.* Individuals of this insect, obtained from a laboratory culture maintained at Lincoln University were also used to measure background rubidium levels. A mean rubidium concentration for these wasps was calculated. Any individual collected from the experimental areas which had a rubidium content more than three standard deviations greater than the mean "background" content was considered to be marked (Stimmann, 1974). Using this threshold, if the rubidium content in unmarked parasitoids is normally distributed, the chance of classifying an unmarked insect as marked would

be 1 in 769. Here, the assumption of normality was tested using the Kolmogorov-Smirnov test. The laboratory culture of *D. tasmanica* was started from insects reared from leafroller larvae collected from Canterbury vineyards; these larvae had passed through 3-4 generations in laboratory culture before being used in this study.

To measure the dispersal of *D. tasmanica* from the buckwheat, 30 sticky traps were placed at distances of 0, 4, 10 and 30 m in both directions from the buckwheat in each vineyard area on the day after each of the three RbCl applications and were left in the vineyard for seven days. The traps were hung from the wires used to support the grape vines and therefore, traps placed at 0 m were placed at less than 20 cm from the buckwheat flowers. On the final trapping date, the buckwheat in one of the five areas had died and therefore traps were not erected in this area. The traps were removed for *D. tasmanica* identification (J. Berry, HortResearch, Auckland, pers. comm.) and individuals of *D. tasmanica* were carefully removed from the traps using a fine paintbrush, dipped in white spirit and they were then placed individually in separate microcentrifuge tubes and stored at -20°C until they were analysed for their rubidium content.

To determine whether the buckwheat had been marked with rubidium and the extent to which concentrations had declined over time in these plants, the concentration of rubidium in the plants was measured by collecting leaf samples for nine days following the third RbCl application. Approximately 10 leaves were collected from randomly-selected buckwheat plants in each of the four areas in which the buckwheat persisted. The leaves were then placed in polythene bags and stored at -20°C for later analysis. Leaves from untreated buckwheat grown in a glasshouse at Lincoln

University were collected to test for the background rubidium concentration in buckwheat.

4.3.4 Sample preparation and rubidium analysis

Individual *D. tasmanica* were dried in an oven at 30-35°C for 24 hours and then weighed on a microbalance for the later determination of whether the amount of rubidium in individual *D. tasmanica* was related to the size of the wasp. After weighing, a two-step wet-oxidation method described by Corbett et al. (1996) was used to digest the wasps. Following digestion, 40 µl of each sample was placed in a 2 ml sample cup, to which 1560 µl of deionised water was added. Three aliquots of 20 µl each were analysed for each sample, so 1/80 of each wasp was analysed for its rubidium content.

Rubidium concentrations in *D. tasmanica* were measured using a GBC Scientific GF 3000 graphite furnace with a PAL 3000 Auto Sampler and a rubidium lamp at a wavelength of 780 nm. Program parameters were 700 °C for 20 s charring and 2500 °C for 1 s of atomisation. Quantification of the rubidium content for each individual wasp was accomplished using analytical grade rubidium chloride as a standard. Standard rubidium solutions of 0, 2, 4, 6, 8 and 10 ppb of rubidium were analysed according to the expected range of samples and were used to calculate the amount of rubidium in each sample.

Buckwheat leaf samples were dried in paper bags in an oven at 30-35 °C for 2-3 days. The samples were then crushed to a fine powder and weighed on a microbalance and a 0.5 g sample was sub-sampled for analysis. The sub-samples were then added to

digest tubes and 10 ml of concentrated nitric acid was added to each tube. Tubes were then placed in a heating block and heated for 30 min at 40 °C, 2 hours at 80 °C, 2 hours at 125 °C and then for 2 hours at 140 °C. The contents of each tube were transferred to a 25 ml volumetric flask, ensuring that the tube contents were rinsed with deionised water. Each flask was then topped up with deionised water to 25 ml and samples were transferred to a 30 ml sample jar and stored in a refrigerator until the rubidium analyses were conducted. The rubidium content of the buckwheat samples was analysed using a flame atomic absorption spectrophotometer (GBC Avanta). Standards of 1, 2, 3, 5 and 10 ppb of rubidium were used to calculate rubidium levels in the buckwheat plant samples.

4.3.5 Parasitism rates of leafroller larvae by *D. tasmanica*

To measure parasitism rates at different distances from flowering buckwheat, eight randomly selected vines were searched for larvae at distances of 0, 4, 10 and 30 m from the flowers in each vineyard area on 18 March and 6 April, 2004. Vines were selected using random numbers and each vine was searched for six minutes, as this was the average amount of time required to find the greatest number of leafroller larvae per vine (Scarratt, unpublished). Leafroller larvae found were placed into a tube containing diet (adapted from Singh, 1977) and reared at 20°C ± 2°C, with a 16L: 8D photoperiod or until they developed into an adult moth or a parasitoid. The latter were identified to species. To calculate parasitism rates, both collection dates and all vineyard areas were combined.

4.3.6 Statistical analyses

The number of rubidium-marked wasps collected was too few to perform statistical analysis and therefore, the mean number of rubidium-marked wasps was calculated. Data for the proportion of leafroller larvae parasitised by *D. tasmanica* was analysed using generalised linear models. To investigate the nature of the trend between the proportion of leafroller larvae parasitised and the distance from the buckwheat, linear and quadratic trends were fitted. There was no correlation between the rubidium concentration in each wasp and the weight of *D. tasmanica*; therefore parasitoid weight was removed from any further analyses.

4.4 Results

4.4.1 Background levels of rubidium

Unlabelled adult *D. tasmanica* from the laboratory culture and the field contained an average of 0.961 ± 0.816 ng of rubidium per wasp (mean ± SD, n = 21). As the background concentrations of rubidium were normally distributed (D: 0.12, P > 0.15), any individual with more than 3.409 ng of rubidium was considered to be marked (Stimmann, 1974).

4.4.2 Labelling buckwheat plants

The buckwheat plants were successfully labelled with rubidium and the concentration in the leaves did not decay over the nine days for which leaf samples were collected (Fig. 4.1). The mean concentration of rubidium in the buckwheat leaves across all the areas seven days after RbCl had been applied at 2000 ppm was 1140 ± 319 μg/g. The rubidium had been absorbed by the plants and remained at relatively high concentrations one week following spraying.

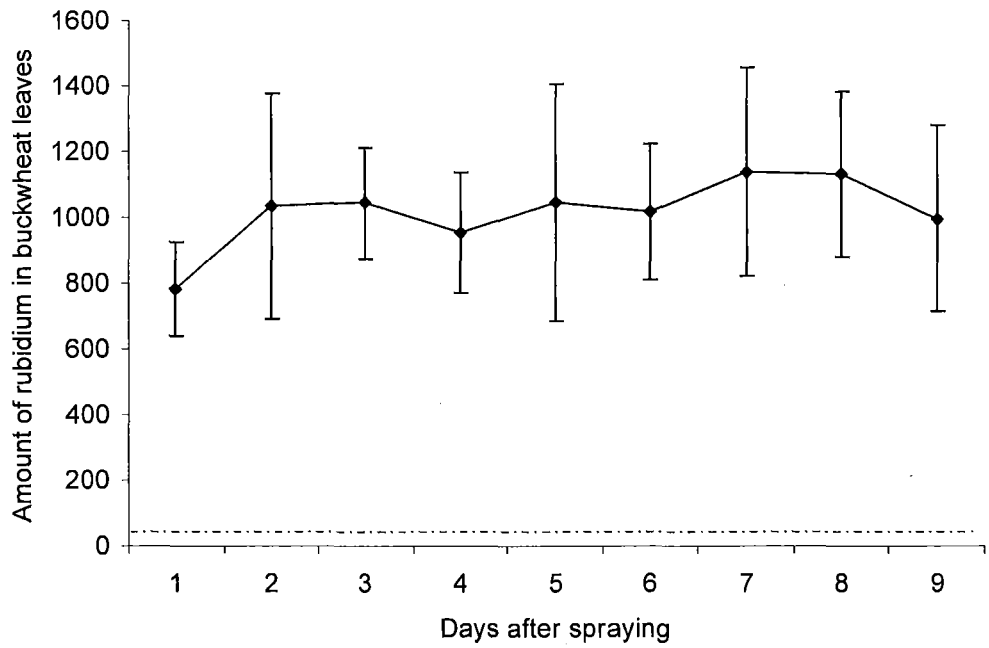

Fig. 4.1 Mean (± SE) amount of rubidium (μg/g) in the buckwheat leaves nine days following the spraying of 2000 ppm RbCl onto the buckwheat. The dotted line (···) indicates a background level of rubidium in buckwheat leaves.

4.4.3 Dispersal of marked *D. tasmanica* from buckwheat

213 *D. tasmanica* were collected on the traps over the three collection dates. Similar numbers of *D. tasmanica* were caught at each of the distances sampled (Fig. 4.2). Of these, 65 were marked with rubidium. The results showed that there were fewer marked female *D. tasmanica* caught than males (Fig. 4.2) and that both male and female *D. tasmanica* dispersed up to 30 m in a seven-day period. The number of marked male *D. tasmanica* collected was similar at all distances from the buckwheat, with less marked male *D. tasmanica* being collected at 10 m (Fig. 4.3). The number of marked females did not differ at increasing distances from the buckwheat (Fig. 4.3).

However, so few female *D. tasmanica* were collected that this result might be influenced by the small sample size.

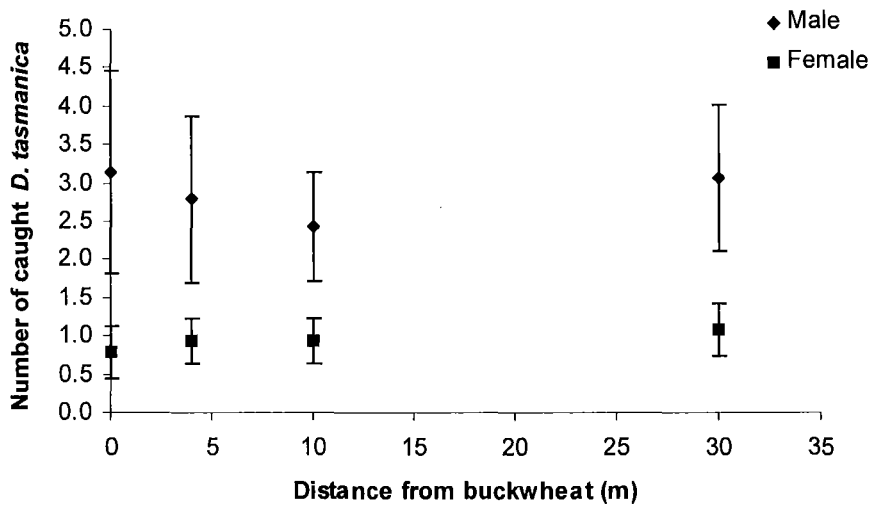

Fig. 4.2 Mean (± SE) number of male and female *D. tasmanica* caught on traps at increasing distance from the buckwheat.

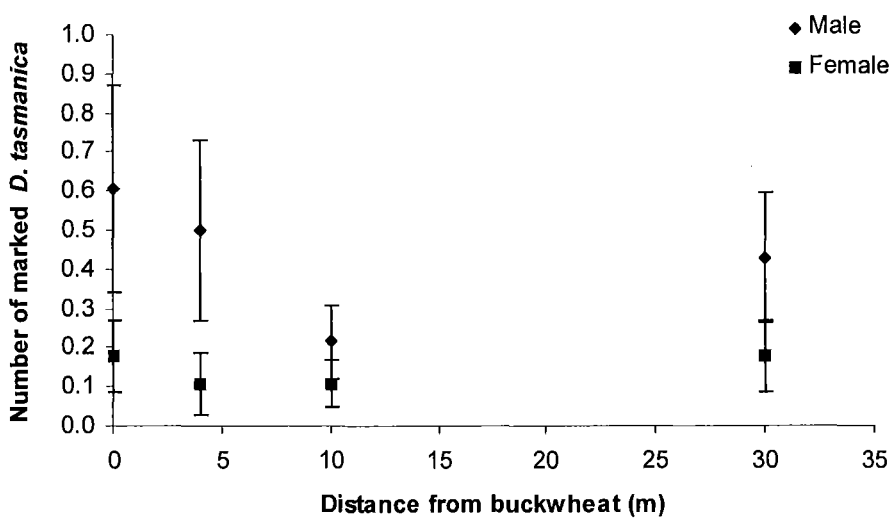

Fig. 4.3 Mean (± SE) number of rubidium-marked male and female *D. tasmanica* collected at increasing distance from the buckwheat.

4.4.4 Parasitism rates

The proportion of leafroller larvae parasitised showed evidence of a quadratic trend with increasing distance from the buckwheat (df = 1, P = 0.023; Fig. 4.4). However, there was no reason why parasitism rates should be lower at 4 and 10 m from the buckwheat than at 0 and 30 m from it and therefore it was considered that parasitism rates were relatively uniform at all distances from the floral resources (Fig. 4.4).

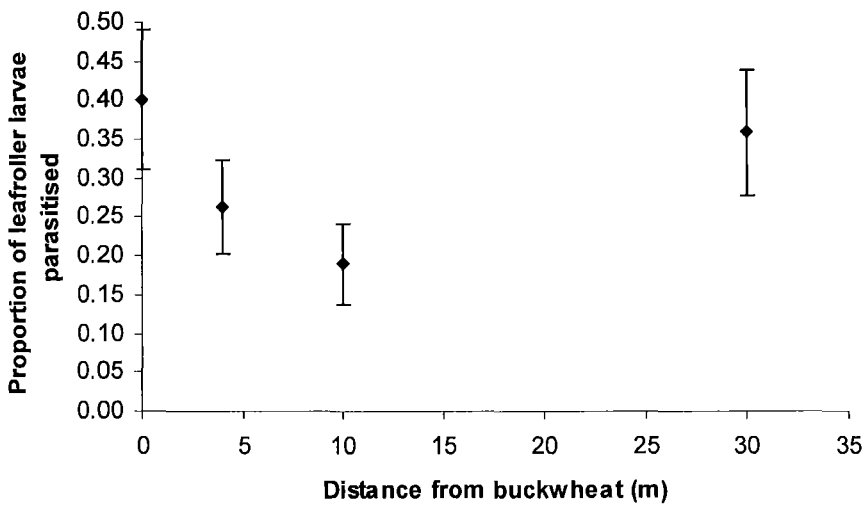

Fig. 4.4 The proportion (± SE) of leafroller larvae collected that were parasitised by *D. tasmanica* at increasing distance from buckwheat.

4.5 Discussion

With the exception of work by Freeman-Long et al. (1998), Wratten et al. (2003) and Schellhorn et al. (2004), there has been little published research on the dispersal of natural enemies from floral resource subsidies in agricultural systems. More specifically, there has been no work on the dispersal of *D. tasmanica* and yet there is information on the dispersal of its main host (Suckling et al., 1994). In the present study, the dispersal of *D. tasmanica* from flowering buckwheat was examined and the results showed that individuals marked with rubidium were found up to 30 m from the flowers within a seven-day sampling period. Also, the number of rubidium-marked male and female *D. tasmanica* did not differ with increasing distance from the buckwheat. However, as few female *D. tasmanica* were caught on the traps, and fewer were marked, it is difficult to comment on their distribution and dispersal from the buckwheat.

Results of the present study indicate that buckwheat can be marked with rubidium following foliar applications of RbCl solutions and that high concentrations of the element can be maintained in the leaves more than nine days following spraying, suggesting that there was minimal decay of rubidium over this time. Gu et al. (2001) sampled and analysed the nectar of plants that had been watered with RbCl solution and showed that it could also be marked in this way. Therefore, in future studies using rubidium as a self-marking technique, analysing both the nectar and the parasitoids would be valuable. It would ensure that the nectar had been marked with rubidium and would provide further evidence that the parasitoids had been feeding on the nectar and that they were not marked as a result of being on the plants at the time of spraying, or through the acquisition of rubidium from their host larvae. Previous

research has shown that *E. postvittana* larvae may feed on buckwheat leaves when no other food source is present, although they do not mature successfully on it (Irvin et al., in press). Therefore it is unlikely that, in the present study, rubidium was passed on to *D. tasmanica* via the emergence of the parasitoids from their marked hosts. Further research could also examine whether homopteran honeydew can be marked with rubidium. If this were possible, this technique could be used in conjunction with HPLC to determine where parasitoids had been feeding and whether parasitoids were feeding on nectar, honeydew or both of these sugar sources (Heimpel et al., 2004).

Finally, the trapping method used to collect *D. tasmanica* in this study may have influenced the results, as some traps (especially coloured ones) may be more attractive to a particular cohort of parasitoids. For example, yellow traps may be attracting "hungry" parasitoids, compared with those which have recently fed on buckwheat and may explain why so few rubidium-marked parasitoids were caught. In the present study, 30% of *D. tasmanica* caught on the traps were marked with rubidium, indicating that they had fed on the buckwheat; however, if a large number of "hungry" parasitoids were attracted to the traps, this percentage may be an underestimate of the number of parasitoids which had actually been feeding on the buckwheat. Also, coloured traps may attract parasitoids of a certain age. For example, a gravid female parasitoid may have sought out nectar resources when it was an immature adult, but may be more likely to seek hosts rather than nectar in its later life (Takasu & Lewis, 1995). For this reason, its responsiveness to coloured traps may be lower at this stage. Finally, there is some evidence that yellow traps may be more attractive to male insects during periods of reproductive activity (Chandler, 1985; Horton, 1993), as males may spend more time searching for food whereas females

may spend more time searching for hosts. Therefore, egg-laden females may be less dispersive. In the present study, at least three times as many rubidium-marked male *D. tasmanica* were caught on the traps than were females; however, this may not be an indicator of the true population sex ratio. Consequently, parasitoid behaviour in the presence of floral resources may differ depending on the sex of the parasitoid and /or its physiological state (Wäckers, 1994; Hickman et al., 2001; Jervis et al., 2004) and these factors as well as the type of trap used to catch parasitoids must be taken into consideration when studying parasitoid behaviour in relation to floral resources.

Berndt et al. (2002) showed that *D. tasmanica* abundance was increased when buckwheat flowers were present in vineyards and showed that parasitism rates of leafroller larvae by *D. tasmanica* could be increased adjacent to buckwheat flowers (Berndt, unpublished data). However, there has been little published work on the "distance of effect" of floral resource subsidies on parasitism rates of insects (Tylianakis et al., 2004), including of leafroller larvae by *D. tasmanica*. The results of the present study show that parasitism rates were greater adjacent to the flowering buckwheat and that these rates decreased with increasing distance, up to 10 m but then parasitism rates were similar at 30 m to parasitism rates adjacent to the buckwheat (0 m). These increased rates of parasitism close to the buckwheat may be as a result of greater aggregation of parasitoids near the floral resources (Berndt et al., 2002) or as a result of increased fecundity as a result of feeding on the nectar from these plants (Berndt & Wratten, 2005). However, as there were not significantly higher numbers of female *D. tasmanica* near the buckwheat, nor more rubidium-marked females near these flowers, interpretation of the current results is difficult. One reason why there was no difference in the number of marked female *D.*

tasmanica between distances from the buckwheat was probably that the number of females collected was too low to detect a difference. High numbers of rubidium-marked males did however occur near the buckwheat and as males were much more abundant than females, this supports the sample size interpretation. In this study, the proportion of leafroller larvae parasitised at 30 m was greater than at 10m. One possible reason for this might be that at 30 m larvae were parasitised by a separate cohort of *D. tasmanica* that had not visited the buckwheat. Finally, although the results of this study showed greater parasitism rates close to the buckwheat than at 10 m from it, a reduction in pest (leafroller) populations could not be demonstrated. Landscape-scale processes are likely to be operating (Thies & Tscharntke, 1999); adding functional biodiversity at the vineyard or landscape scale could address this, although there are social, political and economic factors influencing this (Griliches, 1960; Rogers, 1983). As parasitoids may be dispersing further from floral resources than originally thought when this study began, it may have been appropriate to sample at distances greater than 30 m.

In this study buckwheat plants were effectively marked with elevated levels of rubidium, *D. tasmanica* were marked with rubidium after feeding on the buckwheat flowers, the dispersal of *D. tasmanica* from these floral resources was measured and parasitism rates of leafroller larvae by *D. tasmanica* was measured. Therefore, it is concluded that using rubidium to mark parasitoids following feeding on floral resources is an effective technique which may be used to study the feeding ecology and dispersal of insect natural enemies and the distance over which floral resource subsidies may be acting in agro-ecosystems.

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Chapter 5 The effects of buckwheat on parasitism rates and abundance of leafroller larvae in a large-scale field experiment

5.1 Introduction

Biological control is the use of natural enemies to maintain pest populations at lower densities than would occur in their absence (DeBach, 1964; Hajek, 2004). Quantifying natural enemy and pest population dynamics is one way of determining the impact that natural enemies are having on pest populations (van Driesche & Bellows, 1996). This may include determining natural enemy abundance, the evaluation of natural enemy efficacy, via the assessment of parasitism rates in naturally-occurring hosts or artificially deployed ones and pest density or abundance (van Driesche, 1983; van Driesche & Bellows, 1996). Each one of these parameters can provide a measure of whether the natural enemy, or in this case the habitat manipulation practice, is having an effect on the population dynamics of the pest.

In New Zealand, Stephens et al. (1998) first investigated the effects of floral resources on parasitism rates of leafroller larvae by *D. tasmanica*. In that study, parasitism rates of naturally-occurring leafroller larvae were investigated in an orchard and they were higher in buckwheat areas (34 %) compared with controls (20 %). When Irvin et al. (in press) measured parasitism rates of artificially released leafroller larvae in orchards, rates were significantly higher in the alyssum and buckwheat treatments compared with the control. However, parasitism rates of naturally-occurring leafroller larvae did not differ between treatments, possibly due to a low number of replicates. In contrast, Berndt et al. (2002) found no difference in parasitism rates of artificially released leafrollers by *D. tasmanica* in buckwheat and control plots in vineyards and

subsequently no difference in the number of larvae on vines or in bunches. However, when parasitism rates of naturally-occurring leafroller larvae were examined in New Zealand vineyards, buckwheat increased parasitism rates of larvae in one of the three vineyards only (from 18 % to 45 %) (Berndt et al., in press). This finding may have been due to the low numbers of leafroller larvae in the vineyards during the year that this study was conducted or perhaps also because insecticides had been sprayed in two of the three vineyards. This study illustrates some of the possible problems associated with working in agricultural systems where insecticides are used as common practice. Overall, these studies indicate that buckwheat is having some effect on parasitism rates of leafroller larvae but they do not provide a clear indication of whether buckwheat is consistently increasing parasitism rates of leafrollers by *D. tasmanica,* nor have these studies shown reductions in pest densities in the field. Also, these studies were conducted at small scales and as *D. tasmanica* can disperse at least 30 m in one week (Chapter 4) a larger scale study was necessary to determine whether buckwheat could have an effect on parasitism rates of leafrollers by *D. tasmanica,* without concerns of movement between the experimental treatments.

Therefore, in the present study, the effects of buckwheat on parasitism rates of naturally-occurring and released leafroller larvae were examined and the abundance of leafroller larvae on grape vines and in grape bunches were measured in a large-scale vineyard experiment. These results are discussed in terms of the management of leafrollers in New Zealand vineyards and recommendations are made for future research in this area.

5.2 Materials and methods

5.2.1 Study site

Six areas of grapevines (cv. Sauvignon Blanc) were selected at Seresin Estate, an organic vineyard in Marlborough, New Zealand for use in this study (Fig 5.1). Of these six areas, three areas were randomly selected to be (blocks B, E and F) sown with buckwheat into cultivated ground in every sixth inter-vine row on 15 November 2004 (the buckwheat flowered from 30 December until 19 February) and 25 January 2005 (the buckwheat flowered from 21 February until 6 April) at 45 kg/ha to promote continual flowering throughout the season. Three areas (blocks I, G and J) were randomly selected and were maintained as controls, where no flowering plants were planted and the vineyard floor consisted of grasses and clovers. The dimensions of the blocks were; block B, 200 m by 90 m, block E, 125 m by 75 m, block F, 180 m by 150 m, block I, 200 m by 180 m, block G, 200 m by 95 m and block J, 150 m by 75 m.

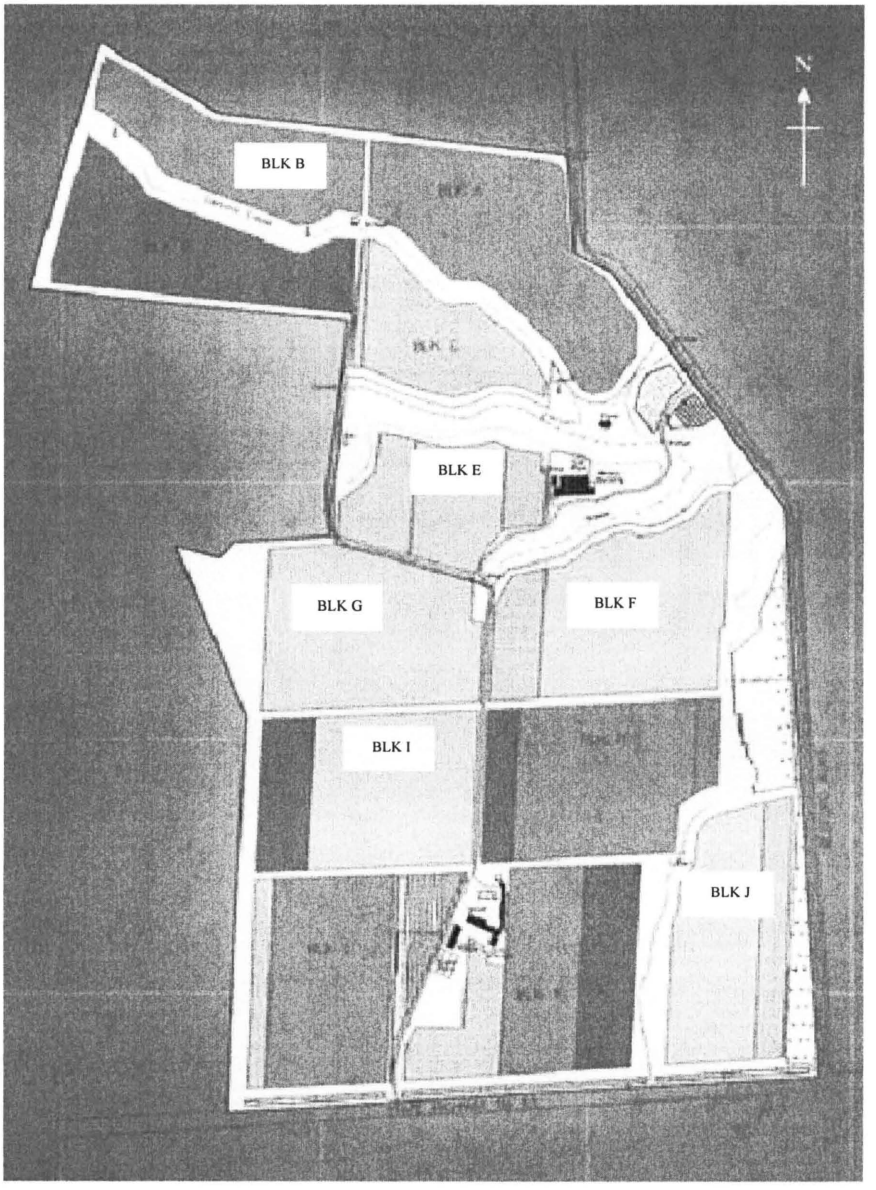

Figure 5.1 Layout of experimental blocks at Seresin Estate, 2005.

5.2.2 Parasitism rates and abundance of naturally-occurring leafroller larvae

To measure parasitism rates of leafroller larvae by *D. tasmanica* and larval abundance, leafroller larvae were collected from vines on seven dates throughout the season; 4 January, 25 January, 7 February, 21 February, 7 March, 21 March and 4 April 2005. On each of these collection dates, five rows were randomly selected in

each of the six vineyard areas and in each of these rows four randomly selected vines were searched for larvae, so that a total of 20 vines were searched in each experimental area on each date. Vines were searched for six minutes, as this was the amount of time required to collect the greatest number of larvae in the least amount of time (see Fig. 3.1). Any leafroller larvae that were found on the vines were collected and placed in tubes containing larval diet (Singh, 1983) and tubes were labelled with the date and location of collection and were sealed with cotton wool. Larvae were reared in temperature-controlled rooms at 20 ± 2 °C and with a 16L: 8D photoperiod until an adult moth or a parasitoid emerged. Parasitoids were identified to species, where possible. A small number of larvae that were collected died whilst being reared and were excluded from the subsequent analysis.

Naturally-occurring leafroller larvae in grape bunches

On 7 March, 21 March and 6 April 2005, 100 grape bunches in each of the six vineyard areas were searched for leafroller larvae. Any larvae that were found in bunches were collected and were placed separately in tubes containing larval diet (Singh, 1983) and were reared in the same conditions as described above until an adult moth or a parasitoid emerged. The average number of larvae collected per bunch was calculated and was compared between buckwheat and control treatments.

Statistical analysis

The percentage of leafroller larvae that were parasitised by *D. tasmanica* was analysed using a generalised linear regression model (GLM) with binomial proportions. Predicted values from the model were used to plot the percentage of leafroller larvae parasitised by *D. tasmanica* in each treatment. The number of

leafroller larvae collected on vines and in bunches were analysed separately using a generalised linear regression model, with a Poisson distribution. Predicted values from the model were used to plot the number of leafroller larvae collected in bunches.

5.2.3 Parasitism rates of released leafroller larvae

To examine parasitism rates of leafroller larvae by *D. tasmanica* at different host densities, larvae were placed in the vineyard on potted grapevines on seven occasions throughout the grape growing season; 23 January, 4 February, 18 February, 3 March, 18 March, 4 April and on the 8 April 2005. Grapevines (cv. Sauvignon Blanc) were propagated from canes collected from Canterbury vineyards. Vines were grown in ten-inch pots with a 3-4 month soil mix and vines were used in the experiments when more than ten leaves were present on each vine. Vines were seeded with high or low densities of 1-2 day old *E. postvittana* larvae; these larvae were maintained on the vines for five to seven days and then the vines were placed in each of the six vineyard areas described previously. On 23 January, 4 February and 18 February, six vines were each seeded with 20 larvae and six with 100 larvae. On 3 March, the number of larvae seeded on each vine was decreased as it was thought that 20 and 100 larvae per vine was an unrealistic density of larvae in the vineyard, so, 12 vines were each seeded with five larvae and 12 were each seeded with 10 larvae. As the number of recovered larvae from the 3 March seeding was low, the number of seeded larvae was increased. So, on 18 March, 4 April and 8 April, 12 vines were each seeded with 10 larvae and 12 with 20 larvae. On 23 January, 4 February and 18 February, one vine of each larval density was placed in each of the six vineyard areas. The potted vines were at least 40 m apart. On 3 March, 18 March, 4 April and 8 April, two vines of each larval density were placed in each of the six vineyard areas, as above. The

seeded vines were each placed in a 20 litre bucket half filled with water to prevent the soil in the pots from drying out. They were maintained in the vineyard for seven days, after which time the vines were removed from the vineyard and each was searched for remaining larvae. These were collected, placed in a tube containing larval diet (Singh, 1983) and reared in a temperature-controlled room at 20 ± 2 °C and with a 16L: 8D photoperiod until an adult moth or a parasitoid emerged. Parasitoids were identified to species, where possible.

Statistical analysis

As few larvae were recovered from the vines and of those that were recovered, some died whilst being reared, statistical analysis could not be conducted on the data. Therefore, the percentage of leafroller larvae that were parasitised by *D. tasmanica* was expressed as;

$$\left[\frac{\text{Number of leafroller larvae recovered parasitised by } \textit{D. tasmanica}}{\text{Number of larvae recovered} - \text{number of larvae that died}}\right] \times 100$$

The percentage of larvae parasitised was compared between buckwheat and control treatments.

5.3 Results

5.3.1 Parasitism rates and abundance of naturally-occurring leafroller larvae

The number of leafroller larvae collected on vines did not differ between treatments ($F = 0.06$, $df = 1$, $P = 0.810$; Fig. 5.2). The number increased throughout the season until 21 March.

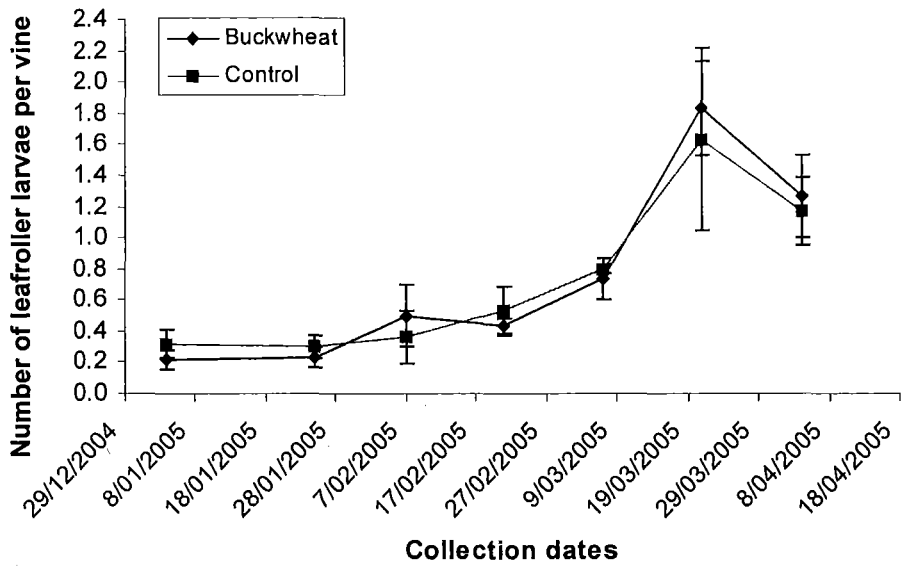

Fig. 5.2 Mean (± SE) number of larvae collected per vine in buckwheat and control treatments.

Parasitism rates of naturally-occurring leafroller larvae

Significantly more leafroller larvae were parasitised by *D. tasmanica* in the buckwheat than in control areas ($F = 89.33$, $df = 1$, $P < 0.001$; Fig. 5.3). Also, the date on which the larvae were collected had a significant effect on parasitism rate ($F = 2.57$, $df = 6$, $P = 0.017$; Fig. 5.4), as the percentage of leafroller larvae parasitised in the buckwheat treatment increased from January to February, then again from March to April (Fig. 5.4).

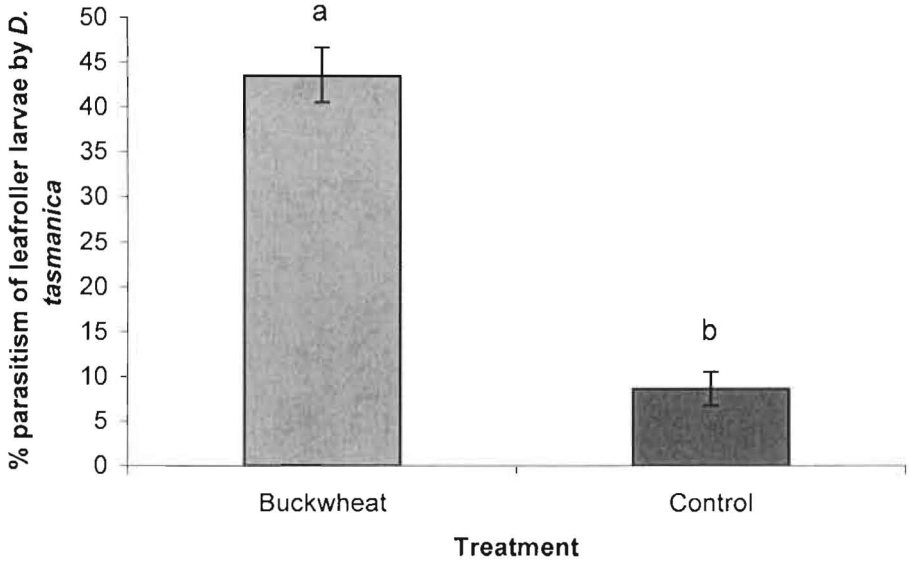

Fig. 5.3 Mean (± SE) percentage parasitism of leafroller larvae by *D. tasmanica* in the two treatments (collection dates combined). Mean values used were predicted values from the GLM. Significant differences are indicated by different letters ($P < 0.001$).

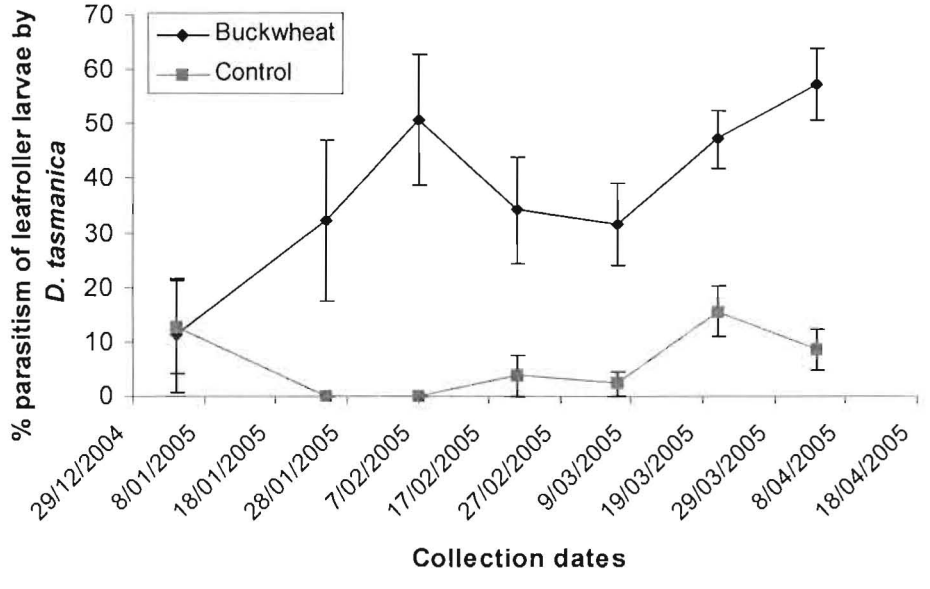

Fig. 5.4. Mean (± SE) percentage parasitism of leafroller larvae by *D. tasmanica* in buckwheat and control treatments. Mean values used here were predicted values from the GLM.

Naturally-occurring leafroller larvae in grape bunches

When all collection dates were analysed together, there was a significant effect of treatment on the number of leafroller larvae collected in bunches ($F = 4.00$, $df = 1$, $P = 0.046$). When the number of leafroller larvae collected in bunches was analysed separately for each collection date, there were no significant differences in the number of larvae collected in bunches in buckwheat and control treatments on 7 March ($F = 0.04$, $df = 1$, $P = 0.835$) or 21 March ($F = 0.05$, $df = 1$, $P = 0.827$). However, significantly more larvae were collected in bunches in the control treatment than the buckwheat treatment on 6 April ($F = 8.15$, $df = 1$, $P = 0.004$; Fig. 5.5).

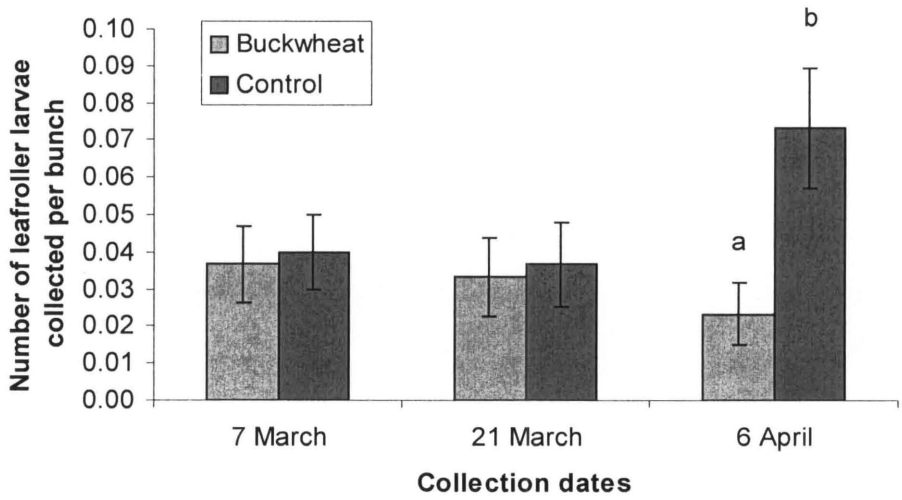

Fig. 5.5 Mean (± SE) number of leafroller larvae collected per bunch in buckwheat and control treatments. Mean values used here were predicted values from the GLM. Significant differences between treatments are indicated by different letters ($P = 0.004$).

5.3.2 Parasitism rates of released leafroller larvae

Although the percentage of released leafroller larvae parasitised by *D. tasmanica* could not be analysed statistically, parasitism rates of larvae were greater in areas where buckwheat was planted compared with control areas for all larval densities (Table 5.1). Except for when five larvae were seeded on vines, percent parasitism appeared to increase with increasing larval density (Table 5.1). However, the percent parasitism did not differ much between when 20 or 100 seeded larvae and this may be as a result of larval densities being extremely high and above those that would be found in the field. Finally, parasitism rates appeared to be lower on the vines seeded with a 10 larvae compared with 20 larvae, perhaps as 10 larvae per vine is closer to natural densities.

Table 5.1 The mean percentage of larvae parasitised by *D. tasmanica* in buckwheat and control treatments.

	Number of larvae seeded on vines					
Treatment	5	10	10	20	20	100
Buckwheat	75	25	63	86	85	85
Control	35	0	22	60	53	62

5.4. Discussion

5.4.1 Parasitism rates and abundance of naturally-occurring leafroller larvae

Greater parasitism rates can be expected to lead to decreased pest densities under most conditions if the nectar source has no direct effects on pest numbers (Heimpel & Jervis, 2005). However, the number of larvae on vines did not differ between the buckwheat and control treatments even though parasitism rates of the larvae in buckwheat areas was much higher than in control areas. A reason for this may be the high fecundity of *E. postvittana* (Danthanarayana, 1975) and also that *D. tasmanica*-induced mortality, even via the provision of floral resources cannot overcome the effects of high pest fecundity. This is also thought to be the reason why predation by ladybird beetles (Coleoptera: Coccinellidae) may not reduce the numbers of mealybugs (Hemiptera: Pseudococcidae) (Dixon, 2000). Another possible reason why reductions in the number of leafroller larvae were not observed on grapevines may be due to the adult leafroller moths being highly dispersive (Suckling et al., 1994) and therefore movement between the treatments and from non-vineyard areas may have counteracted any effects the treatments were having on larval mortality. Also, although a similar number of leafroller larvae were collected in buckwheat and control areas, nearly 50% of the larvae in the buckwheat areas were parasitised and would have resulted in death of the larvae, compared with less than 10% in the control areas. Therefore, there would have been almost 40% fewer larvae becoming late-instar larvae and subsequently adults in the areas where buckwheat was planted compared with control areas. Finally, reductions in pest abundance may not have been recognised, as when the larvae were collected individuals from different generations were not distinguished. In Marlborough, leafrollers generally exhibit three generations per year (Lo & Murrell, 2000) and therefore if parasitism of leafroller larvae increases

as the season progresses (as shown in Fig. 5.4), it is likely that fewer later-generation early-instar larvae were collected in the buckwheat treatment later in the season but this was masked by late-instar larvae left over from the first generation being collected at the same time. Further work could address this.

Parasitism of naturally-occurring larvae by *D. tasmanica* was significantly greater in areas of the vineyard where buckwheat was planted compared with control areas. Also, parasitism rate in buckwheat areas increased from December to February and then decreased slightly until March when it increased again until sampling ceased in April. These fluctuations in parasitism rates by *D. tasmanica* may reflect the lifecycle of this parasitoid species; where emergence of adults occurs in December – January (refer to Chapter 3; abundance of *D. tasmanica* on traps) from overwintering leafroller larvae.

When naturally-occurring larvae were sampled in bunches on three dates, significantly more were found in bunches in control areas but on 6 April only. One possible reason why larval abundances did not differ in bunches on the first two sampling dates may be as late-instar larvae were more commonly found in bunches than were early-instars and that 6 April coincided with the third generation of *E. postvittana* becoming late-instar larvae and therefore, moving into bunches. As a greater percentage of larvae was parasitised by *D. tasmanica* in buckwheat areas, larvae in control areas are more likely to become late-instar larvae (as they have largely escaped parasitism) and move into grape bunches. This result is important as in New Zealand the number of infested grape bunches at harvest time is one of the

thresholds that grapegrowers use for determining whether control of leafrollers is required (Charles, 2002).

5.4.2 Thresholds

It is recommended that insecticides should be sprayed in spring (before flowering) if more than 5 % of grape bunches had been infested with leafroller larvae during harvest of the previous year (Charles, 2002). These thresholds are based on work done in the wine-growing region of Hawke's Bay, New Zealand in Chardonnay grapes (Lo & Murrell, 2000). In this study, the authors found that the timing of leafroller infestations had a major influence on the type of damage to grape bunches, where early infestations caused the greatest direct losses because with only small berries present, larvae fed mainly on stalks, killing whole groups of berries. Later in the season larvae fed more on mature berries and damage became confined to fewer fruits but disease became a more important factor. They also made the association that grape bunches infested with larvae had higher incidence of disease and a yield loss of 12 % was estimated per bunch infested with leafroller larvae. Even though the authors stressed the need for caution when using these results to set thresholds in other grape varieties (as different varieties have different susceptibility to disease (Nicholas et al., 1994)) and across different years (as this study was conducted in a dry year when disease was low), this damage level has been recommended to set thresholds for leafrollers across New Zealand. Another problem with using the damage level from this study to set thresholds is that damage levels in different grape varieties will equate to differing amounts of financial loss. For example, in New Zealand, Chardonnay grapes at the time of this study were worth $1200/t, whereas Sauvignon Blanc grapes from Marlborough are currently worth in excess of $2000/t. Finally, in

their study, Lo & Murrell (2000) infested bunches with two larvae each to determine yield loss, so if infestations were more likely to be only one larva per bunch, the amount of damage caused might be less than the predicted 12% and consequently thresholds would need to be adjusted accordingly. Despite the much-needed work conducted by Lo & Murrell (2000), there is a real need for further work to measure the economic damage caused by leafrollers in wine grapes in New Zealand so that the unnecessary spraying of insecticides in vineyards may be prevented. Also, further work could address whether the number of bunches infested with leafrollers near harvest can be reduced to below economic thresholds by the presence of buckwheat in the vineyard as in this study, only the total number of larvae collected per bunch was measured and not the percentage of bunches infested.

5.4.3 Parasitism rates of released leafroller larvae

Although very few of the released larvae in the current work were recovered, parasitism of larvae by *D. tasmanica* was higher on vines that were placed where buckwheat was planted. When larvae were seeded on vines at 20 or 100 per vine, parasitism rates were far greater than those on naturally-occurring larvae. When larvae were seeded on grapevines at 10 larvae per vine, parasitism rates more closely represented those on naturally-occurring larvae. It is likely that this response of higher parasitism rates when greater densities of *E. postvittana* larvae were seeded on vines is evidence of a density-dependent aggregative numerical response (Hassell, 1978; Hajek, 2004) and not just as a consequence of parasitoid concentration near the flowering buckwheat.

5.4.4 Leafroller management in New Zealand vineyards

The results of the present study clearly demonstrate that buckwheat can increase parasitism of leafroller larvae in New Zealand vineyards. This increase in parasitism may contribute to fewer larvae in bunches close to harvest, therefore reducing the need to spray insecticides the following spring. However, it may be necessary to examine the effects of floral resources on predators of leafrollers as well as the effects on a greater species complex before buckwheat is planted throughout New Zealand vineyards. Also further work could address whether flowering buckwheat can reduce leafroller populations over several generations. This question may be usefully explored further with the support of ecological modelling (Kean et al., 2003).

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Chapter 6 Discussion

In New Zealand, research on enhancing the natural enemies of leafrollers using floral resource subsidies has spanned nearly a decade, with the work beginning in orchards and then moving into vineyards. Prior to the current study, the research provided important information on the effects of certain floral resources on *D. tasmanica* and other natural enemies of leafrollers. However, the results differed between studies and were not consistent across years. Therefore, this study aimed to rank buckwheat against other flowering plant species to determine whether there are any other species which may also enhance the biological control of *E. postvittana,* to determine the spatial scale over which nectar sources affect parasitoid dispersal and to determine the effectiveness of buckwheat in enhancing the biological control of *E. postvittana.*

6.1 Levels of success reached using CBC to manage leafrollers

As discussed in Chapter 1, there exists a hierarchy of research outcomes which researchers hope to achieve when conducting CBC research (Gurr et al., 2003). The first step in the hierarchy is to increase natural enemy abundance near flowering plants. In Chapter 3, *Glyptapanteles* spp. and *Glab. stokesii* abundance was increased when flowering buckwheat was added to the vineyard system, thus the first step in the hierarchy was achieved. Also, more *D. tasmanica* were found in buckwheat than in control areas, but this result was significant on one date only.

The second step in the hierarchy is to enhance natural enemy 'fitness' and this was demonstrated in Chapter 2, where buckwheat flowers enhanced the longevity and fecundity of *D. tasmanica* in laboratory experiments. Also, the fitness of the pest, *E.*

postvittana was not enhanced by exposure to buckwheat plants, nor was buckwheat the "preferred" feeding substrate by young *E. postvittana* larvae when feeding was compared between grapevine leaves and leaves of the flowering plants tested. This was an important finding as it is crucial to screen for a 'selective food plant' that would benefit the parasitoid without benefiting the pest (Baggen & Gurr, 1998).

The third step, an increase in parasitism rate of the pest was demonstrated in Chapter 3, but as this experiment was conducted only in one vineyard area, it was decided that examining parasitism and the fourth and fifth steps in this hierarchy (reducing the pest and reducing the pest to below economic thresholds) should be done in a large-scale field experiment. Chapter 5 showed that parasitism rates of leafroller larvae by *D. tasmanica* were greater in areas of the vineyard where buckwheat was planted (44%) compared with control areas (9%). Also, fewer leafroller larvae were found in grape bunches near harvest in buckwheat compared with control areas. However, the second part of the fifth step, reducing the pest below economic threshold, was not measured as economic thresholds for this pest are based on percentage of bunches infested by leafroller larvae (Charles, 2002). In this study, the number of larvae in bunches was measured. This is something that could be further pursued, because if planting buckwheat can reduce larvae in bunches, it may be able to reduce the percentage of bunches infested with larvae in areas where buckwheat is grown. If this is the case, grape growers may be more likely to take up this technology and implement it in their vineyards.

The parasitoid nectar provisioning hypothesis states that biological control of pests will be improved through the presence of nectar-producing plants that supply

parasitoids with sugar (Heimpel & Jervis, 2005). However, unlike the hierarchy of research outcomes (Gurr et al., 2003), this hypothesis suggests that it is not parasitoid aggregation which is important, but that it is more important to validate that the mechanisms leading to nectar-mediated improvement of biological control actually occur in the field and that the magnitude of the effect is enough to drive pests below economic thresholds. In this study, *D. tasmanica* fed on fructose in the vineyard (presumably from the nectar of buckwheat flowers). In laboratory experiments, *D. tasmanica* fecundity was enhanced after exposure to buckwheat flowers and in the vineyard, increased rates of parasitism occurred in areas planted with buckwheat. Despite these positive results, pest densities in the presence of nectar were decreased in bunches on one date only. As this is the final step in the nectar provisioning hypothesis (Heimpel & Jervis, 2005) and the hierarchy of research outcomes (Gurr et al., 2003), more work needs to be done to examine further whether the use of flowering buckwheat in the vineyard can lead to overall reductions in the pest.

To ascertain the optimal spacing required for floral resources to be planted in the vineyard, the distance that *D. tasmanica* dispersed from flowering buckwheat was measured (Chapter 4). Although measuring how far natural enemies disperse after feeding on floral resources is not a requirement of the hierarchy of research outcomes (Gurr et al., 2003) nor of the nectar provisioning hypothesis (Heimpel & Jervis, 2005), it can provide valuable information to growers on what spacing floral resources may be planted in agro-ecosystems. The results outlined in Chapter 4 showed that *D. tasmanica* disperses at least 30 m from flowering buckwheat in one week. Also, parasitism rates of leafroller larvae were greater closer to the buckwheat flowers and decreased with increasing distance from them (up to 10 m), which may be

an indicator of aggregation near the flowers or enhanced fitness as a result of nectar feeding. Future work could further examine the dispersal of female *D. tasmanica* from flowering buckwheat plants, as few females were caught in this study and as a greater understanding of the spatial dynamics of female *D. tasmanica* could enable the further enhancement of the biological control of leafrollers. Also, parasitoid behaviour in relation to floral resources could be an area of study worth pursuing as the trade off between host and food searching by natural enemies in the field is only beginning to be understood (Siekmann et al., 2004) and could be further explored in this system.

6.2 The management of leafrollers in New Zealand vineyards

As the results of this study and previous studies (Berndt et al., 2002; Berndt et al., in press) have demonstrated, incorporating floral resources into vineyards can enhance the biological control of leafrollers. For that reason, it would be expected that grape growers would use this technology to manage leafrollers, either exclusively or as part of an integrated pest management programme, especially as the negatives associated with pesticide use are well documented (Hajek, 2004). However, the adoption of this technology is not common in New Zealand vineyards (Shadbolt, 2005) and when grape growers were asked why they were not using biological control techniques as part of their vineyard management, the main reasons given were the possible risks involved and labour costs (Shadbolt, 2005).

Therefore, even though there are positives associated with the use of floral resources in vineyards, there is still a need to demonstrate to grape growers that the costs involved with implementing alternative methods of pest control can outweigh the financial costs associated with the losses of crop as a result of pest induced pressure

and/ or the financial and social costs associated with pesticide use. To do this, economic thresholds for leafrollers need to be reviewed (as mentioned in Chapter 5), as these thresholds do not reflect the current market value of wine grapes and may be leading to the unnecessary spraying and overuse of pesticides.

6.3 The New Zealand wine industry

The New Zealand wine industry is expanding rapidly, with sales of New Zealand wine estimated at over one billion dollars by 2010 (Gregan, 2005). The question regarding this growth is how will the industry maintain its image of producing wines from the "riches of a clean, green land", especially when wine growing in New Zealand is so dependent on chemical input? Continued reliance on the frequent use of chemical inputs is unsustainable and problems associated with this approach include pesticide resistance and the suppression of natural enemies such as predators and parasitoids (Theiling & Croft, 1988). Biological control practices, such as conserving natural enemies through the use of floral resources in vineyards to enhance the biological control of insect pests should be included as part of an integrated pest management plan for New Zealand vineyards. By using such techniques the New Zealand wine industry may maintain this sustainable farming image, so that wines made in New Zealand can continue to be produced from terroir that is "clean and green".

6.4 Future research

As stated earlier, there is a need to determine whether leafroller populations can be reduced by the use of floral resources in vineyards. Further work could also address the impact of predatory insects on leafrollers, examine ways of enhancing such

predators and also examine the effects of floral resources on non-target insects such as hyper-parasitoids and predators of natural enemies. Also, there has been very little work which has investigated whether floral resources can enhance the fitness of natural enemies in the field and this deserves further research, as many studies, including this one, have assumed that enhanced fitness in the laboratory will lead to enhanced fitness in the field.

6.5 Conclusions

Overall, the results of this study indicate that exposure to buckwheat leads to increased fitness of *D. tasmanica* in the laboratory, increased rates of parasitism of leafrollers in the vineyard and fewer leafroller larvae in bunches at harvest. However, showing reductions in the pest population as a result of increased parasitism is difficult to achieve in CBC research and only further study can determine whether this is occurring in the *D. tasmanica*–leafroller vineyard system. Finally, the next step in managing leafrollers in vineyards in New Zealand is to incorporate the use of buckwheat into an integrated pest management plan, where the technologies developed as part of this project may be used successfully with others to manage these pests.

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